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THE DEVELOPMENT OF ROOT-KNOT NEMATODE GALLS

J. R. CHRISTIE

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INTRODUCTION

Many nematodes penetrate the tissues of growing plants for the purpose of securing food. Some species in the process of feeding move about through the plant, causing considerable destruction of cells, and may appropriately be regarded as "grazers." In such cases abnormal structures frequently result that usually are referred to as galls. These are brought about in part by irregularities of growth resulting from tissue destruction and in part by cellular hypertrophy. In other cases a highly developed interrelationship has been evolved between the plant tissues on the one hand and the nematode on the other, and both host and parasite react in a definite and characteristic manner. The so-called root-knot nematode, *Heterodera marioni*,¹ is an example of this type of highly specialized, obligate parasite.

Over 900 different species of plants, including both monocotyledonous and dicotyledonous, are known to serve as hosts for the root-knot nematode. The parasite usually penetrates such underground parts of the plant as roots, rhizomes, and tubers, where it induces the development of abnormal growths or enlargements. In some instances the basal portion of the stem is invaded. The size and character of these enlargements vary in different plants. In the case of *Gerbera* and, to a less extent, *Cyclamen*, external manifestations are sometimes small and inconspicuous or even lacking, while in the case of *Thunbergia laurifolia* and rhubarb, enormous structures nearly 2 feet in diameter may develop. It is interesting to note that the unusually large galls reported by Steiner, Buhner, and Rhoads (14) developed at the base of the stem.

The adult female parasite usually lies with its head imbedded in the vascular cylinder, the posterior part of the body extending into the cortex. The eggs, after deposition, are surrounded by a gelatinous substance. As they accumulate at the posterior end of the parasite (Fig. 8, C) the cortical tissue is often ruptured and the eggs are pressed out and may occur in masses at the surface of the root. Two kinds of infestation take place.

¹ *Heterodera marioni* (Cornu, 1879) Goodey, 1932; synonym, *Heterodera radiculicola* (Greeff, 1872) Müller 1884 of authors. Not *Anguillulina radiculicola* (Greeff, 1872) Goodey, 1932; synonym, *Anguillula radiculicola* Greeff, 1872.

1. Eggs may hatch within the root and the larvae migrate into adjacent tissue where they become established. This type of infestation is evidently of common occurrence, often resulting in the formation of unusually large galls.

2. Eggs may hatch at or near the surface of the root and the larvae escape into the soil. These larvae migrate to, and enter, new roots. The present paper deals exclusively with galls produced by this type of infestation.

It has been demonstrated repeatedly that at or near the root tip is a favored point of entrance. However, penetration of the larvae evidently is not confined to this region. Galls occur on rhizomes, tubers, and various parts of the plant under circumstances that render unlikely the entry of the parasite at a growing point. Nevertheless, a very large percentage of the galls found on most plants is initiated by larvae that penetrate near the tips of growing roots.

About 60 to 72 hours after the larva has become established in the root tissues, cells lying adjacent to the head of the parasite begin to undergo a change. They increase in size, the nucleus divides and there are finally formed the peculiar, multinucleate structures, long known in the literature as giant cells.

LITERATURE

Müller's excellent paper (9) is the earliest comprehensive study of the morphology and development of *Heterodera marioni*. The structure and development of the galls also are discussed in a general way, those that occur on *Musa rosacea* being selected as examples. This investigator noted the proliferation of cells in the region outside the vascular cylinder and the disarrangement of the vascular elements, but failed to mention the giant cells.

Frank (5), in his study of gall development, secured his material from various cultivated plants including clover, *Trifolium pratense*; beets, *Beta vulgaris*; lettuce, *Lactuca sativa*; pears, *Pyrus communis*; and teasel, *Dipsacus fullonum*. The writer notes that, in general, cells in the region of invasion retain their ability to divide for a considerable time. There is formed around the region of the central cylinder a layer of short, thin-wall, cells, some of which may later be differentiated into xylem elements. With regard to this Frank writes as follows: "Die Folge ist, dass, wenn in dem umliegenden meristematischen Gewebe einzelne Zellen zu getüpfelten Holzelementen werden, dieselben so wie ihre Mutterzellen eine mehr kurze Form besitzen und diese Zellengruppen unregelmässig verschoben liegen, so dass ihre Zellen auf dem Querschnitte in den verschiedensten Richtungen zur Längsausdehnung durchschnitten erscheinen." Frank further notes that the walls of cells adjacent to the cavity occupied by the parasite usually remain thin. No mention is made of giant cells.

Treib (17) briefly describes the effects of *Heterodera marioni*, which he

erroneously named *H. jarvanica*, on the roots of sugar cane. This investigator mentions that the larvae are able to enter roots with but slight injury to the cells. He also notes a tendency for lateral roots to occur at the region of the gall, but believes this results from larvae migrating to regions where lateral roots are forming. Treub describes and figures the giant cells. Commenting on the division of their nuclei he writes, "Jamais je n'ai rencontré jusqu'ici dans les grandes cellules, des noyaux présentant des phases de la division indirecte. Par contre, j'ai vu plusieurs fois — — —, où les noyaux font l'effet de se multiplier par division directe."

Atkinson (1) studied the life-cycle of *H. marioni* and the effect of its presence on the roots of various plants including cotton, tomato, potato, and parsnip. He reports that the parasites are not confined to any particular tissue element or system, but locate at various points in the root, including the central cylinder, cambium, parenchyma, or even the bark. The mature female cyst often is protected by only a thin layer of dead peripheral tissue or may even be exposed. Atkinson reports that all the tissue elements of the diseased root undergo hypertrophy and some are subject to special changes in form and direction of growth. Parenchyma cells whose tangential diameter is normally greater than their radial, are so changed that their radial diameter is greater, a change that seems to take place in nearly all the parenchyma of the gall, whether near the cyst or distant from it. No mention is made of giant cells nor does Atkinson describe or figure any structures that can be recognized unmistakably as such.

Queva (12) studied the effect of *Heterodera marioni* on the roots of *Dioscorea illustrata*. The parts of the root system selected for study were the nonfleshy portion that connects the fleshy roots with the main root system, and the fleshy roots themselves. In the first instance the parasites locate at the periphery of the primary vascular cylinder. In regions not occupied by galls the vascular cylinder is composed of numerous, radially arranged, primary vascular bundles ("faisceau multipolaire"). In a series of transverse sections, as one approaches the gall, secondary vascular tissue appears. This is formed by a group of cells in the pericycle located on the outer margin of each primary xylem bundle. The innermost cells of each of these groups, those adjacent to the primary xylem bundle, become differentiated into secondary xylem, while the outermost cells become differentiated into phloem. There are thus developed bundles of secondary vascular tissue forming a more or less continuous layer surrounding the vascular cylinder. Near the middle of the gall the vascular tissues of the central cylinder, according to Queva, are no longer recognizable as such, the cell walls not being characteristically thickened. The secondary vascular tissue in this region is separated from the primary vascular cylinder by several layers of newly formed cells and it is from these that the giant cells are derived.

Beille (2) studied the structure of galls on the roots of *Papaya gracilis*. Here the parenchymatous tissue within the gall divides rapidly, forming a mass in which are embedded the giant cells. The cortex sloughs off and the endodermis and pericycle become unrecognizable. The giant cells are limited exteriorly by vascular tissue of new formation, in which the phloem is reduced, but the xylem, composed of short reticulate elements, is more abundant. According to this author, the walls of the giant cells disintegrate and allow the protoplasmic contents of adjacent cells to intermingle. Of special interest is his statement that the body of the parasite, or cyst, is enclosed in 8 or 10 layers of suberized cells.

The galls studied by Molliard (8) were from the roots of "Melon," *Coleus* and *Begonia*. This writer notes that, after invasion, the root tip may be arrested in its growth and also that lateral roots frequently develop near the region of invasion. In the melon the giant cells are surrounded by a layer of small, thin-wall cells that, in certain regions, eventually become thick-wall xylem elements. The number of such regions corresponds to the number of primary vascular bundles in the normal root. Molliard notes the irregularities in the form and number of giant-cell nuclei and their subsequent coalescence and disintegration. He also discusses the function of giant cells in supplying food to the parasite.

Tischler (16), studying galls on the roots of *Circaea lutetiana*, devoted his attention primarily to the cytological aspects of giant-cell formation, particularly the methods of nuclear division. He concluded that during early stages of development the division of giant-cell nuclei is by normal mitosis. After a certain period, however, there follows division by amitosis and by fragmentation.

For information regarding the formation and structure of the giant cells we are especially indebted to Němec (10). According to this investigator, inward migration is intercellular, the larvae never boring through cells, a fact that accounts for the absence of dead cells in the region of their path. As long as a larva remains in the periblem no further changes take place in the root cells. As soon as the head is inserted into the plerome the cells close to the mouth opening of the larva immediately commence to enlarge, their plasma content increases and their nuclei divide, without, however, the formation of separating walls. There are formed, therefore, early in the development of the gall, multinucleate giant cells. Only cells of the plerome undergo this transformation.

Němec is inclined to attribute the formation of giant cells to the stimulating action of some substance secreted through the mouth of the parasite. He considers the possibility of its being brought about through the withdrawal of substances by the nematode but regards this as unlikely as other parasites withdraw substances from plant cells, as for example fungi, without stimulating the formation of giant cells.

Němec finds that the division of giant-cell nuclei takes place by mitosis. Following nuclear division, but early in the life of a giant cell, nuclei may coalesce. Various stages of this phenomenon were observed. In some cases nuclei were fusing in pairs or, in the case of older cells, all the nuclei might be massed together near the center of the cell. Thus the older the cell the fewer nuclei it is likely to contain. Němec regarded it as probable that cases interpreted by Tischler as nuclear division by amitosis or by fragmentation were, in reality, stages of nuclear coalescence.

Kostoff and Kendall (6), working with galls on the roots of *Nicotiana* hybrids, find that in the early stages of invasion multinucleate cells may be formed by nuclear division without accompanying cell division. However, they believe that a coalescing of cells following dissolution of cell walls may be more generally responsible for the formation of giant cells. This process becomes more and more evident with the continuation of parasitism. Cell walls near the oral region of the parasite become increasingly thinner until finally the content of the cells is removed.

These investigators contend that a secretion produced by the salivary (esophageal) glands of the parasite increases permeability in plant tissues, which results in an exosmosis and brings about an accumulation of food in the region of invasion. "As a consequence of the presence of this accumulation of nutrition the growth of the plant tissues is accelerated in these regions and is morphologically expressed by the swelling, or gall, on the root. At the same time however, the other organs of the plant are deprived of nutrition and their growth is inhibited. The increased permeability is instrumental in bringing about a localization of the foreign substances but the plant suffers a loss of nutrition in the substances so carried from the surrounding tissues to the area of invasion, while the parasite receives a great store of nutritive material in a very small region about its head, as is shown in the rich granular cytoplasmic contents in these regions."

The *Chromatinballung* of Tischler and the *Kernverschmelzung* of Němec, according to these authors, is brought about by agglutination accompanied by proteolytic processes. Conformations referred to by Tischler and others, as due to *Amitotische Zweiteilung*, *Knospung*, *Sprossung* or *Fragmentation*, are probably early stages of nuclear fusion. This is in agreement with the opinion of Němec.

Cook, in a recent paper (4), discusses the development of root-knot nematode galls on tomato, tobacco, and coleus. He compares his interpretations with those of Atkinson and, like the latter, makes no mention of giant cells.

Saran (13) recently has published a brief account of deformities on the roots of *Hibiscus esculentus* in India caused by *Heterodera marioni*. The tendency for lateral roots to be formed at the region of invasion is noted, but giant cells are not mentioned.

METHODS

The following observations were made on roots of the tomato that were infested in the laboratory. Seed was germinated in small flower pots containing sand. As soon as the cotyledons emerged and when the radicles were 2 to 4 cm., long the pot was submerged in a pan of water and, by manipulating, the seedlings were floated free from the sand. Each seedling was laid on the bottom of a Petri dish with the tip of the radicle in a small drop of water. Into this drop were placed newly hatched larvae of the nematode. The entire radicle was then covered with fine dry sand, which was moistened afterwards with water. The Petri dishes were then covered and kept at room temperature for 24 hours and then each dish was filled with water and the seedlings floated free from the sand. In order to wash off any parasites that had not yet penetrated, but that might still be adhering to the surface of the root, the seedlings were rinsed in a second dish of water. Except for cases where the root was killed and fixed immediately after being exposed to larvae for 24 hours, the seedlings were planted in flower pots and transferred to the greenhouse. Every precaution was taken to avoid injuring the radicle during manipulation of the seedling.

The exact age of each gall, therefore, was known to within the limits of 24 hours. Where the root was to be killed and fixed within 24 to 48 hours, several larvae were used, usually about 10. In all other cases 2 larvae were used.

ENTRANCE OF THE LARVAE

In every case penetration took place close to the root tip, but, inasmuch as the larvae were placed at this point and to have entered elsewhere would have necessitated migration along the root, no particular significance can be attributed to this fact. Entrance through the root cap and migration to a position back of the growing point occurred frequently.

There is unquestionably a tendency for the larvae to push the cells apart and pass between them (Fig. 1, A). It is overstating the case, however, to say that cells are never destroyed. Instances have been observed, especially in the apical meristem or the region immediately behind it, where cells are clearly being crushed and destroyed (Fig. 1, B). Cases also have been noted where the parasites appear to have passed through cells instead of between them. These occurred in the central cylinder after some elongation of the cells had taken place. The larva, in migrating along the root, had apparently broken down the end walls and passed through a row of these cells. Nevertheless, migration of the larvae is largely intercellular, as Němec states, and instances to the contrary should possibly be regarded as exceptions, although probably of not infrequent occurrence. Certainly, the destruction of cells, when compared with that caused by the passage of many other nematodes, is surprisingly slight.

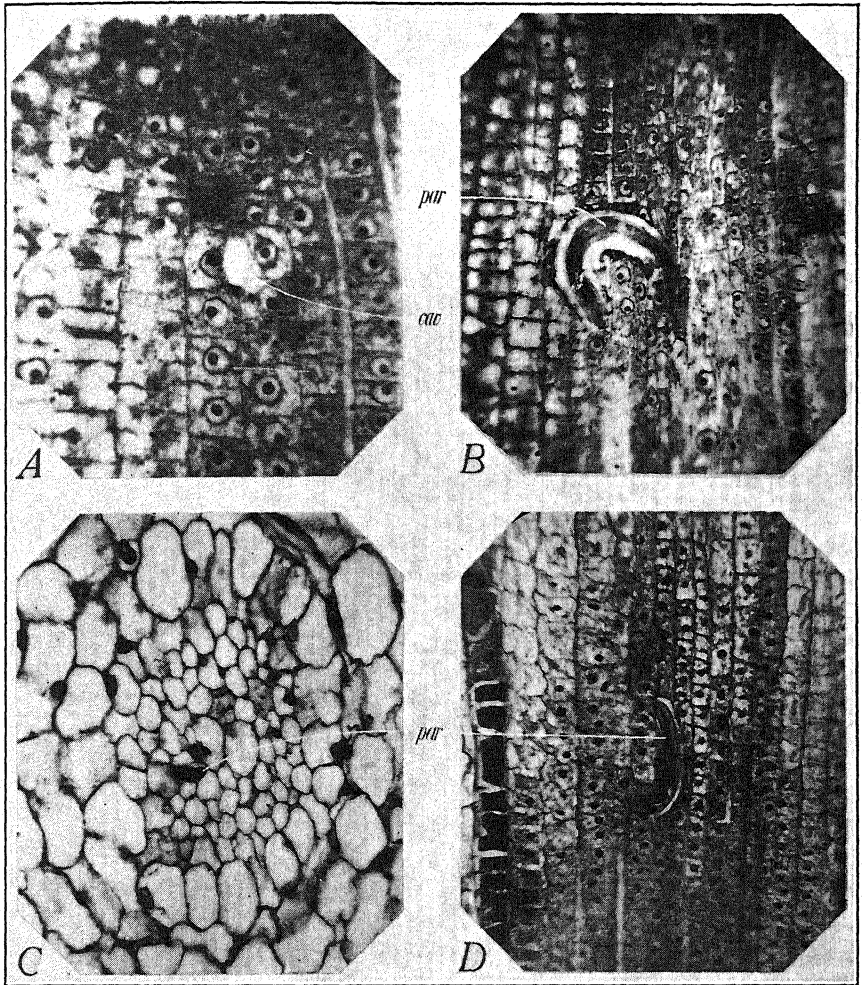


FIG. 1. A. Longitudinal section showing opening (*cav*) caused by migration of larval parasite through root. $\times 400$. B. Longitudinal section showing cell destruction by the entering larva (*par*). $\times 280$. C. Transverse section showing head of larva (*par*) deep in central cylinder. Nearby cells show no noticeable abnormal change. $\times 400$. D. Longitudinal section showing head of parasite (*par*) in plerome at about the beginning of region of elongation. Nearby cells show no noticeable abnormal change. $\times 280$. All sections made after roots had been exposed to infestation for 24 hours.

About all that can be said regarding the final position assumed by the parasite is that its anterior end usually, but not always, lies between the cells of the plerome. Often the posterior end of the body extends into the periblem and, as the root develops, into the cortex. This, however, is not always true, for the entire body of the parasite frequently lies in the plerome parallel with the rows of cells that constitute this region.

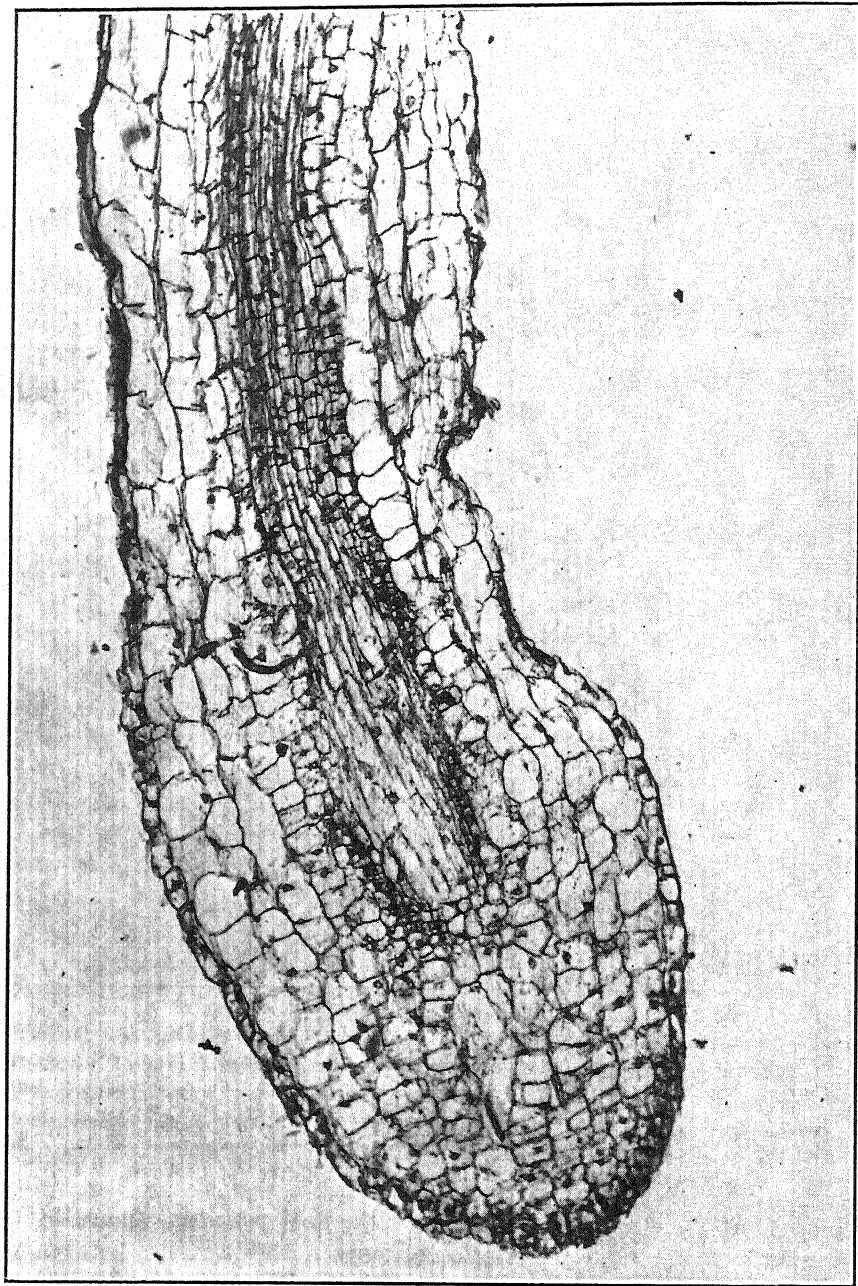


FIG. 2. Longitudinal section through root exposed to infection for 24 hours during which time several parasites entered. The abnormal appearance near the tip is not due to the section's having been cut tangentially or obliquely, as one might at first suspect.
× 138

IMMEDIATE REACTION OF THE PLANT TISSUES

The reaction of the root tissues to the presence of the parasite often is very prompt, although its promptness and extent depend, to some degree, on the number of parasites that enter. The first noticeable change to take place is hypertrophy of the cortical cells. This is not confined to cells lying adjacent to the path of the larva, but those some distance away also may be affected. When several larvae have entered simultaneously, considerable areas in the cortex may show, pronounced cellular hypertrophy at the end of 24 hours. Cells of the pericycle and endodermis, when lying near the path of the larva, will sometimes show slight hypertrophy (Fig. 3, A).

While in many instances a root continues to grow in a more or less normal manner after infestation, sometimes growth is retarded or even stopped, a fact noted by Molliard (8). It is doubtful if the penetration of one larva will ordinarily affect growth to any extent, but when a considerable number enter simultaneously growth may be stopped within 24 hours. The resulting gall will be located, pendulum-like, at the end of the root. Such galls occur frequently in nature.

One root, killed and fixed after being exposed to infestation for 24 hours and harboring several larvae, showed noticeable abnormalities (Fig. 2). Cap cells, for the most part, were lacking. A group of cells near the tip possessed a moderately dense protoplasm, but were vacuolated to some extent and appeared not to be dividing. Cells immediately back of these showed hypertrophy and also were considerably vacuolated. A partly differentiated central cylinder made up of somewhat elongated cells extended far down toward the root tip, where it ended rather abruptly. The surrounding parenchyma cells of the cortex showed hypertrophy to some extent. It would appear, in such cases, that the presence of the parasite suppresses mitotic activity in the apical meristem and hence retards or terminates growth.

One of the first things noticed regarding the galls experimentally produced for this study was the frequency with which lateral roots occurred at the point of infestation. Unfortunately, exact counts were not made, but in fully 60 per cent of the plants from 1 to 4 lateral roots developed in the region of the gall. They were far too common to be explained on the basis of coincidence. This fact has been noted by Treub (16), Molliard (8), Saran (13), and others. Twenty-four hours after infestation one frequently finds, in the region of the parasite, cells of the pericycle undergoing division. In this instance the presence of the nematode seems to stimulate mitotic activity in the pericycle. Once cell division is initiated it proceeds in a more or less normal manner and lateral roots may be formed.

ORIGIN OF THE GIANT CELLS

In the origin of giant cells the first cells affected usually are those of the plerome lying adjacent to the head of the parasite. Larvae frequently be-

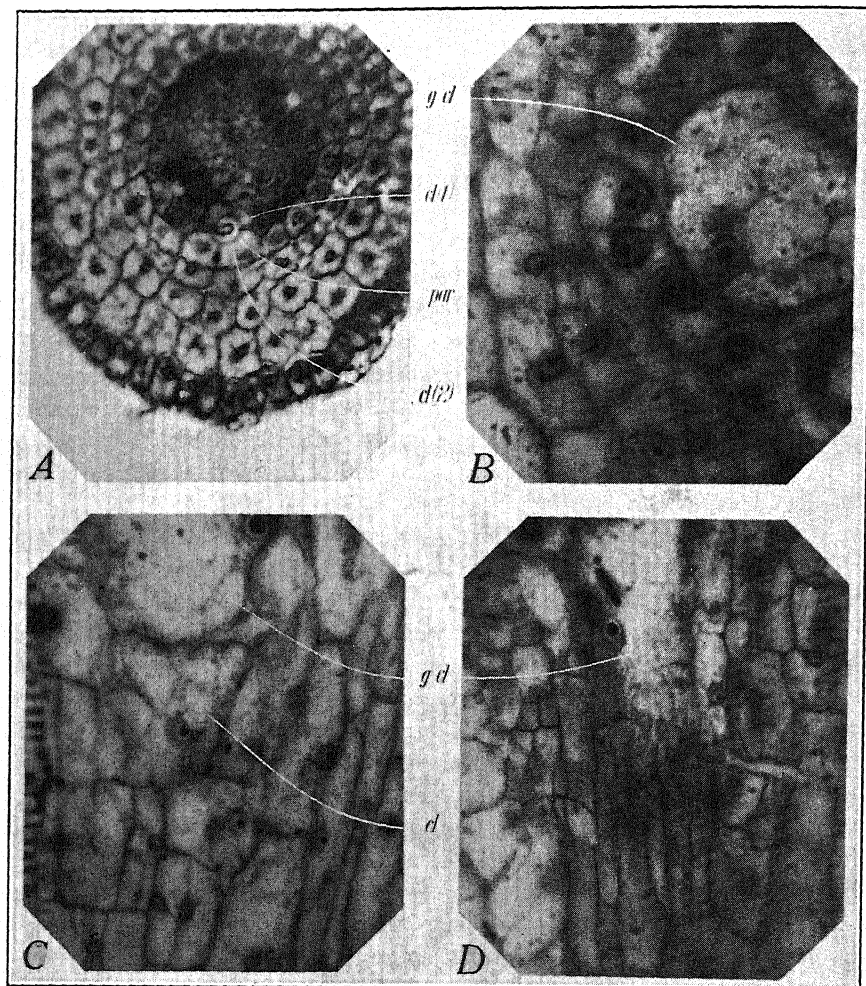


FIG. 3. A. Transverse section through root exposed to infestation for 24 hours. Body of parasite (*par*) is cut transversely where it lies between endodermis and pericycle. In both layers adjacent cells are noticeably enlarged (*cl(1)* and *cl(2)*). Note intercellular position of parasite. $\times 280$. B. Transverse section through 4- to 5-day gall. Lying adjacent to the giant cell (*g cl*) are cells whose nuclei already have assumed the characteristics of giant-cell nuclei. $\times 556$. C. Longitudinal section through 4- to 5-day gall. Cells (*cl*) adjacent to giant cell (*g cl*) are undergoing change, the walls still are fairly distinct. $\times 400$. D. Longitudinal section through 4- to 5-day gall. Cell walls in region adjacent to giant cell (*g cl*) are disappearing. $\times 400$.

come established only a short distance behind the apical meristem. Not all take up this position, as some either enter or migrate farther back. Nevertheless, at the end of 24 hours, larvae frequently are found in the plerome, between the growing point and the region of elongation (Fig. 1, D); and it seems evident, in such cases, that the stimulus that ultimately results in giant-cell formation becomes operative before the cells of the plerome have proceeded far in their normal differentiation.

As the root grows and the cells of the central cylinder elongate some of the cells near the head of the parasite undergo little change. In many instances these are cells that, in normal development, would contribute to the formation of vessels. Such cells elongate but slightly, or not at all, and their walls do not become thickened. Thus, for the first 48 to 60 hours, the stimu-



FIG. 4. Longitudinal section through 8- to 9-day gall. One of the vessels (*vsl*) ends abruptly at a cross wall and the 3 succeeding cells, which would normally have formed its continuation, have remained undifferentiated. One of these cells (*cl*) has divided parallel with the main axis of the root and a delicate separating wall has formed. The body of the parasite is cut transversely (*par*) and the head lies near the point marked *a*, where a giant cell is forming. $\times 150$.

lative effect of the parasite on these cells is to *retard differentiation*. At the end of this period some of these undifferentiated cells begin to enlarge and division of the nucleus may take place. The first division may be parallel to the main axis of the root and a delicate cell wall sometimes is formed (Fig. 4), which, however, soon disappears. The original cell walls also gradually disappear and the protoplasmic contents of adjoining cells coalesce to form the beginning of a giant cell. In the tomato, therefore, giant cells are usually initiated in the vascular cylinder and frequently derived from cells that normally would have taken part in the formation of vessels (Figs. 4, 5, 6, A). However they may occasionally be formed in the cortex (Fig. 6, C) but in such cases the writer has not observed any tendency for them to grow inward and thrust aside the vascular elements as is said to take place in galls formed by *Heterodera schachtii*.

No attempt was made to study the details of nuclear division. This phase of the subject has been more carefully investigated than any other, especially by Tischler (16), Němec (10), and Kostoff and Kendall (6). All agree that nuclear division in early stages of giant-cell formation is by mitosis and there seems little reason to doubt that the amitotic and fragmentation divisions of Tischler were either mitotic divisions of an abnormal character or nuclei in early stages of fusion. Němec mentions the fact that nuclei in the process of division rarely are seen. In this connection it may be pointed out that coalescence of cells takes place at an early stage and, even in a 6- to 7-day gall, a giant cell, in most cases at least, is composed of what was originally several cells. It seems probable that this fact may account for more of the nuclei contained in a giant cell than was hitherto supposed.

DEVELOPMENT OF THE GIANT CELLS

The nuclei of giant cells possess characteristics that differentiate them, to some extent, from nuclei of cells in the corresponding region of a normal root. They are usually large and contain a conspicuous, deep-staining nucleolus. Frequently, 2 nucleoli are present and sometimes, in addition, several other smaller, deep-staining bodies. Occasionally, a nucleolus is lacking, but in its place are many, smaller but distinct, bodies. These conditions apparently are comparable to those described by Tischler (16). In a transverse section of a 4- to 5-day gall one of the first things noted is the prominence of the nuclei in the region occupied by the head of the parasite. This not only applies to giant-cell nuclei but also to those in surrounding cells.

After the coalescing of the first few cells the walls of other cells near the periphery of a giant cell become indistinct. In some places the giant cell has a somewhat definite contour, but at other places there is no distinct line of demarcation between it and adjoining tissue. Adjacent cell walls gradu-

ally break down and the protoplasmic contents of the cells, together with the nuclei, unite with the giant cell (Fig. 3, C & D). Often these nuclei have already assumed the characteristics of giant-cell nuclei (Fig. 3, B). When an area composed of small cells is absorbed a group of nuclei is added that seems out of proportion to the size of the area invaded.

At the beginning of giant-cell formation it is usually several adjacent members of a row of undifferentiated cells in the central cylinder that first coalesce through the dissolution of the separating cross walls. This same tendency persists throughout the early stages of development with the result that giant cells tend to extend longitudinally along the central cylinder rather than laterally into the parenchyma. Nevertheless, the surrounding parenchyma is invaded to some extent; in fact, as noted later, giant cells may be formed exclusively from this tissue (Fig. 6, C).

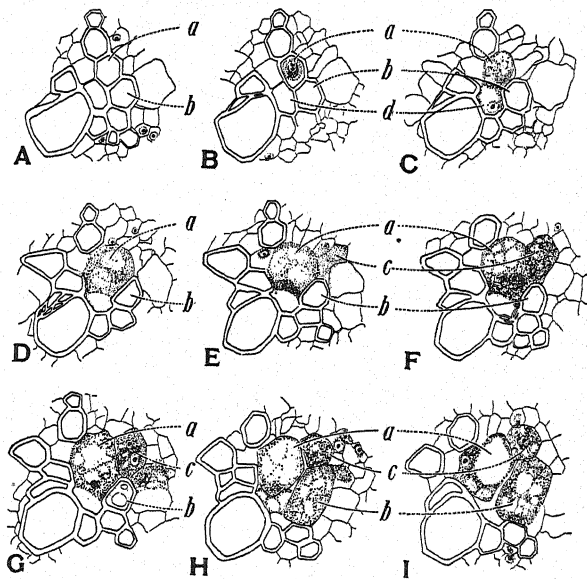


FIG. 5. From a consecutive series of transverse sections, 10μ thick, through 4- to 5-day gall. A. Section farthest from the parasite. Xylem elements *a* and *b* appear normal with thickened walls. B. Xylem element *a* contains a dense protoplasmic mass but the walls are thickened. Xylem elements *b* and *d* appear normal. C. The beginning of a giant cell shows in the place of xylem elements *a* and *d* without a wall to separate the two. Xylem element *b* remains normal in appearance. D. About the same condition as in C; xylem element *b* is normal in appearance. E. A third giant cell (*c*) makes its appearance, apparently having been derived from an adjacent, thin-wall cell. F. Giant cells *a* and *c* are separated by a fairly distinct wall; xylem element *b* remains unchanged. G. This section includes a cross wall in xylem element *b*. H. Xylem element *b* is now replaced by a giant cell derived from cells that normally would have formed a continuation of this element. I. Three fairly distinct giant cells are seen. The head of the parasite has not yet been reached. $\times 230$.

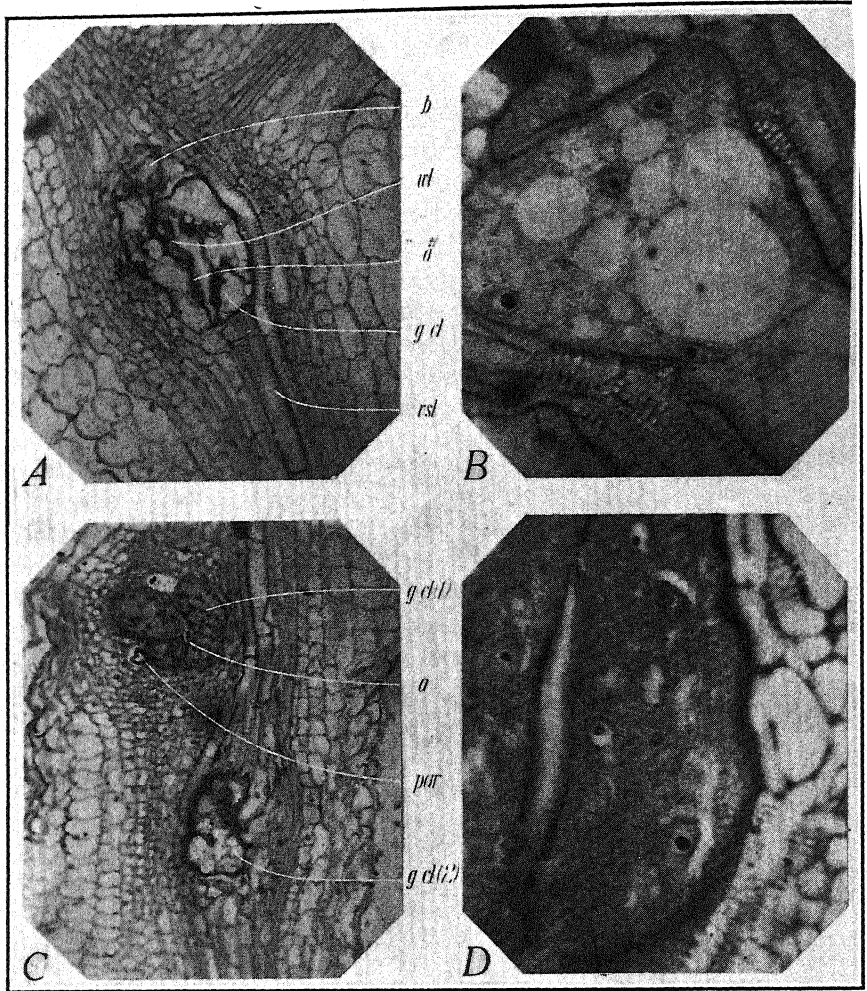


FIG. 6. A. Longitudinal section through 8- to 9-day gall. One vessel (*vsl*) ends abruptly at a cross wall and the cells that normally would have formed its continuation are transformed into a giant cell (*g cl*). Note remnant of cross wall (*wl*). Two more or less distinct giant cells are coalescing at *a*. At *b*, a condition exists similar to that shown in Fig. 3, C, D. A lateral root has developed. $\times 100$. B. Longitudinal section through 10- to 11-day gall, showing typical giant cell. Nuclear membranes are beginning to disintegrate. $\times 280$. C. Longitudinal section through 8- to 9-day gall. Note large number of nuclei in giant cell (*g cl* (1)) formed outside vascular cylinder from small-cell parenchyma as compared with giant cell (*g cl* (2)) formed in vascular cylinder. The parasite (*par*) is cut transversely and the head lies at *a*. $\times 100$. D. Giant cell in a 30- to 31-day gall. Many nuclei have disintegrated. $\times 280$.

Even giant cells of the same age vary considerably in appearance, depending on the character of the cells from which they were derived. Usually they contain, when first formed, large clear areas separated by strands of protoplasm (Fig. 4). As time goes on the protoplasmic network usually becomes denser and the spaces smaller until, at the end of 30 to 40 days, they are more nearly homogeneous in appearance (Fig. 6, D). There is a noticeable tendency, in all stages, for that portion lying close to the head of the parasite to have a dense protoplasm and to stain deeply.

During the first 10 to 20 days the giant cell continues to invade adjacent tissue, coalescing with cell contents after the dissolution of cell walls. As already noted, this does not take place equally in all directions, some areas evidently being more susceptible than others. As the giant cell becomes older this invasion of tissue gradually lessens until, after about 40 days, it appears to have largely stopped.

There are formed in each gall several, usually from 3 to 6, giant cells that remain as more or less distinct units (Fig. 7, D). Giant cells coalesce with the contents of adjacent cells, and, to some extent, with one another (Fig. 6, A), but the writer has never noted an instance, in galls up to 40 days old, where all the giant cells had united to form a single unit, even though they usually lie adjacent to one another.

When examining infested roots at the end of 24 hours it is, of course, impossible to determine whether the position in which the larvae are found is the one in which they would have remained or whether further migration would have taken place. While most of the larvae have the head deep in the central cylinder (Fig. 1, C, D), one occasionally is found with its head in the cortex or between the pericycle and endodermis. That larvae, in some instances, remain in the cortical region, is indicated by the fact that giant cells sometimes are found outside the vascular cylinder (Fig. 6, C).

It has already been suggested that giant cells possess numerous nuclei due largely to the fact that numerous cells contribute to their formation. If this be true it would be expected that where these cells are small and consequently numerous the giant cells would contain a larger number of nuclei than where the cells were large and fewer in number. It is interesting to note, therefore, in the case of a giant cell formed outside the vascular cylinder (Fig. 6, C) from small parenchymatous cells, that the nuclei are very numerous.

REACTION OF ROOT TISSUES SURROUNDING THE GIANT CELLS

Initial stages in giant cell formation take place at about the time when protoxylem is being formed. Outside the central cylinder, and eventually surrounding it, there is a layer of small-cell parenchyma. This, for the most part, originates from the pericycle and outgrowths from it form the

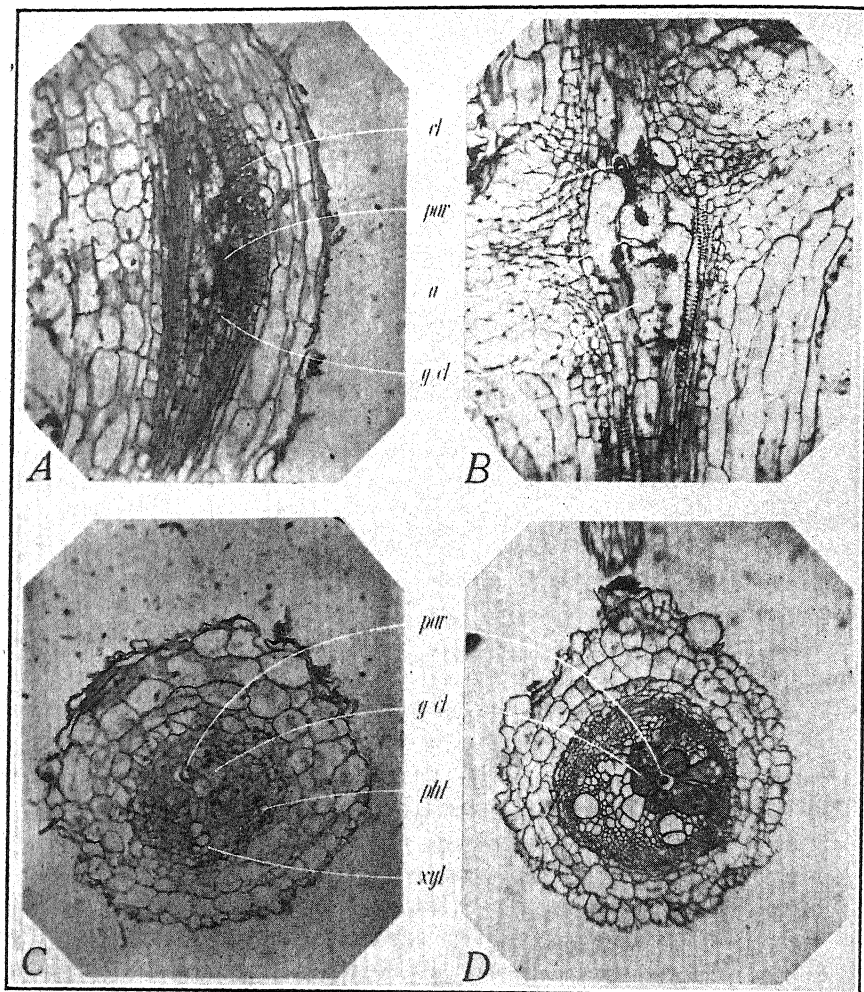


FIG. 7. A. Longitudinal section through 6- to 8-day gall. Note layer composed of small cells (*cl*) bordering the giant cells (*g cl*). $\times 100$. B. Longitudinal section through 7- to 8-day gall. The parasite (*par*) is cut transversely and the head lies near point marked *a*. Two lateral roots have developed. $\times 100$. C. Transverse section through 4- to 5-day gall near head of parasite (*par*). Phloem (*phl*) has formed on both sides in about normal position but xylem (*xyl*) on only one side. On the side of the root where the body of the parasite (*par*) extends into the cortex no xylem has formed. $\times 100$. D. Transverse section through 30- to 31-day gall. Note giant cells (*g cl*) grouped around head of parasite (*par*). $\times 50$.

lateral roots that so frequently develop in the region of infestation. Some of the tracheids or vessels are interrupted in the region of the gall, a fact already noted. Others pass around the giant cells, somewhat pushed out of position, but with their continuity more or less undisturbed. Such continuous vessels, however, usually are few in number. A transverse section through the middle of a 6- to 8-day gall will sometimes show differentiated xylem at both xylem points, frequently at only one and occasionally none at all. Xylem usually fails to differentiate on the side of the root where the body of the parasite extends into the cortex (Fig. 7, C). As the gall becomes older, additional xylem elements are formed. They usually are derived from cells of the surrounding parenchyma. (Fig. 8, B). As these cells are small and irregular in shape, the resulting xylem, as noted by Frank (5), is composed of short, irregular, reticulate elements without direction or recognizable arrangement. It is to this that Beille (2) also refers when he says that the giant cells are limited externally by vascular tissues of new formation in which the xylem is composed of short, reticulate elements.

Usually situated in the vascular tissue, more or less surrounded by xylem, the giant cells eventually come into contact with the thickened walls of these elements. The question arises as to what effect, if any, the giant cell has on these thickened walls. That they are affected, at least to some extent, is indicated by the fact that portions of the walls lying adjacent to giant cells frequently stain differently from the walls elsewhere. Instances were observed also where certain walls of the xylem elements near a giant cell become greatly thickened or swollen. These swollen walls stain a deep red with Flemming's triple stain in contrast to the purplish to blue color usually assumed by such elements. In some cases these modified cell walls blend into the giant cell, as if the substance of the wall were being absorbed (Fig. 8, A). The appearance of such conditions, when seen in stained sections, leads one to suspect that this change is brought about by a substance, produced by the parasite, that seeps between the cells, entering in some places but not in others.

If proteolytic processes result from substances secreted by the nematode, as Kostoff and Kendall (6) suggest, these substances would, when first secreted, occur between the cells. The head of the parasite lies between the cells and there is no evidence that the stylet pierces adjacent walls. Hence cell-wall dissolution may be brought about in this manner, rather than through any direct influence of the giant cell itself.

GENERAL CONSIDERATIONS

One of the first effects of *Heterodera marioni* on root development in the tomato is the suppression, in some instances, of mitotic activity in the apical

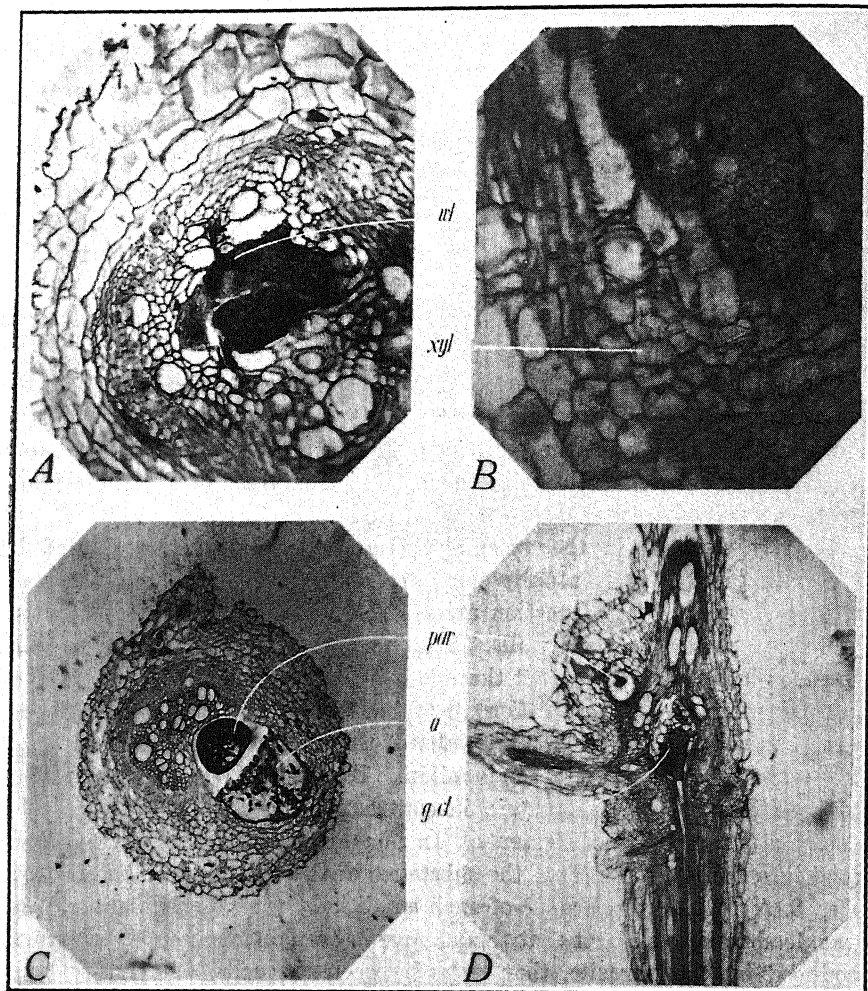


FIG. 8. A. Transverse section through 16- to 17-day gall. In places walls of xylem elements are thickened or swollen and at certain points (*wl*) appear as if undergoing dissolution and being absorbed by the giant cells. $\times 100$. B. Longitudinal section through 10- to 11-day gall showing transformation of parenchyma into small xylem elements. Note formation of reticulate wall thickenings (*xyl*). $\times 280$. C. Transverse section through 35- to 36-day gall passing through posterior end of parasite (*par*) and showing accumulating egg mass (*a*). $\times 50$. D. Longitudinal section through 30- to 31-day gall. Note layer of cortical cells covering cavity in which is seen posterior end of the parasite (*par*), the head of which lies at a point near the giant cells (*g cl*). $\times 25$.

meristem and the consequent cessation of growth. This seems to be at variance with the usual effect of parasites on many plant tissues where extensive cellular proliferation so frequently results. It is also somewhat puzzling that growth of the radicle should be suppressed, while growth of secondary roots in the region of the gall is stimulated. However, in the case of galls on the black oak, *Quercus velutina*, caused by *Andricus punctatus*, Stewart (15) notes that, soon after the gall starts, the cambium at the base of the larval chamber ceases activity, while it is stimulated to greater growth along the sides.

Another effect, manifest soon after entrance of the parasite, is hypertrophy of cortical cells and, to a less degree, cells of the endodermis and pericycle. It is interesting to note that cells of the plerome do not undergo hypertrophy except inasmuch as an increase in size coincident with initial stages of giant-cell formation may be viewed as a manifestation of this phenomenon.

The presence of the parasite stimulates cell division in the pericycle, which results in the formation of a layer of parenchyma not found in normal roots. This tendency for parenchyma to be formed in galls of all kinds is a fact emphasized by Küster (7) who writes "Vor allem ist die Tendenz zur Parenchymbildung auffällig: die Gallen sind fast durchwegs parenchymatische Gebilde." It will be recalled, however, that in the case of these nematode galls some of the parenchymatous cells eventually become differentiated into xylem elements.

Of special interest is the fact that cells of the plerome close to the head of the parasite are at first retarded in their differentiation. This is in harmony with observations on the development of many galls. Wells (18) finds, in the case of "witches-brooms" on *Celtis occidentalis*, induced by *Eriophyes* sp., that tissues in the bases of the gall branches are often markedly inhibited in their differentiation. In fact, the group of cecidia frequently designated as kataplasmas are characterized primarily by a lack of tissue differentiation. Indeed, one is reminded that some animal pathologists believe malignant tumors, in some instances at least, originate from cells that lag behind in their differentiation.

While the dissolution of cell walls in the formation of giant cells, so far as the writer is aware, has not been recorded as occurring in other types of cecidia,² it is probable, nevertheless, that the etiology of nematode galls does not differ, in any fundamental way, from that of galls induced by many other organisms. In the development of plant galls, the source of the stimulus, in most cases, is known. To establish the precise nature of that stimulus has been the baffling problem. There is much evidence indi-

² In galls produced on the apple by *Puceron langiere*, Prillieux (11) records finding multinucleate, parenchymatous cells.

cating that, in many instances at least, some substance produced by the parasite is the stimulating agent involved. This has been widely accepted as an altogether probable hypothesis.

While studying the structure and development of these nematode galls, one is very soon convinced that the stimulus, whatever its nature may be, emanates from the head of the parasite. The point at which initial changes take place and the subsequent arrangement and appearance of the giant cells are such as to leave little doubt in the mind of the investigator regarding the truth of this conclusion. The possibility that excretory materials provide the stimulating substance seems unlikely. Other nematodes live in plant tissues and presumably must excrete. They do not, however, induce the development of giant cells.

The frequent resemblance between callus tissue and the tissues in many galls probably has been a factor in leading plant pathologists to view critically the possible rôle of mechanical injury in inducing gall development. In many instances investigators have regarded traumatic stimuli as an important factor. Referring to galls produced by certain hemipterous insects, Cook (3) makes the unqualified statement that "the modification of the plant tissue to form the gall is purely mechanical, being a continuous effort on the part of the plant to heal the wound produced by the repeated puncturing of the cells by the insect."

In the case of galls produced by *Heterodera marioni* the possibility that mechanical injury is the exciting stimulus seems remote. All who have studied this parasite are agreed that, once its position is established, there is no evidence of mechanical injury to surrounding tissue other than that occasioned by gradual pressure due to growth and reproduction. The stylet is apparently never employed to puncture cells lying adjacent to the head. The parasite lies inert in the plant tissue moving but slightly if at all. The dissolution of cell walls and the progressive spread of the giant cells into regions a considerable distance from the parasite can scarcely be explained on the basis of traumatic stimuli. It is, of course, probable that processes are operative similar to those that usually manifest themselves when a foreign body is embedded in living tissue. The proliferation of cortical cells that tends to form an enclosing wall around the parasite may conceivably be the result of this type of stimulus.

That gall formation may be induced through the removal of substances by the parasite is discussed by Němec (10) who points out that other organisms remove substances from plant tissues without stimulating the production of giant cells or the development of galls. This investigator might have qualified his statement to the effect that some other *nematodes* habitually remove substances from the *rootlets* of plants without inducing such developments.

It is probable, therefore, that these various morphological changes in root development are brought about through the stimulating action of some substance secreted through the mouth of the parasite. All the known facts point very convincingly to such a conclusion.

SUMMARY

The galls studied were produced by experimentally infesting the radicles of tomato seedlings with the nematode *Heterodera marioni*. The age of each gall was known to within the limits of 24 hours and represented a series varying from 24 hours to 40 days. The entering larvae tend to pass between the cells. Injury to the root through cell destruction is slight. When permanently located, the head of the larva usually is in the plerome near the beginning of the region of elongation. The immediate effects on the root cells are: hypertrophy of cells in the cortical region; slight hypertrophy of cells of the pericycle and endodermis when lying near the path of the parasite; a stimulation of cell division in the pericycle; and, frequently, a suppression of cell division in the apical meristem.

During the first 48 to 60 hours, cells of the central cylinder lying in the region of the parasite's head remain undifferentiated. After about 3 days these undifferentiated cells enlarge slightly, their nuclei swell, and their walls disintegrate. The protoplasmic contents of adjacent cells coalesce to form a giant cell. Frequently these are members of rows that normally would have contributed to the formation of a vessel. The giant cell invades adjacent areas and other cells are absorbed after dissolution of cell walls. Eventually, nuclear membranes break down and giant-cell nuclei coalesce and finally disintegrate.

Division of the pericycle, stimulated by the presence of the parasite, results in a layer of small-cell parenchyma, outgrowths of which form the lateral roots that so frequently occur. Eventually, some of the innermost of these small parenchymatous cells become differentiated into xylem elements of irregular shape.

That these changes are induced through mechanical injury, through the removal of substances by the parasite or by the stimulation of excretory products, seems unlikely. It appears probable that these morphological developments in the root are due to the stimulating action of some secretion expelled through the mouth of the nematode.

DIVISION OF NEMATOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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PATHOGENICITY AND VARIATION IN PHYTOPHTHORA SPECIES CAUSING HEART ROT OF PINEAPPLE PLANTS¹

F. P. MEHRLICH

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INTRODUCTION

During the period of commercial pineapple growing in Hawaii, heart rot has been one of the principal causes of failure of young plants. The disease is present to a limited extent in almost every field during the early months of each planting.

In 1930 an extensive study of the disease and of the causal organisms was started by the writer. Its primary purpose was to find an economic control of the disease. Other phases of the study sought to relate heart rot to other diseases of the pineapple plant and of other economic and weed plants; to study the epiphytology of heart rot; to learn more of the physiology and morphology of the pathogens.

This paper presents a record of the world distribution of the disease and its local occurrence in the Hawaiian Islands; discusses the synonymies of the pathogens and presents detailed evidence establishing the synonymy of *Phytophthora cinnamomi* Rands and *Pseudopythium phytophthoron* Sideris; and considers physiologic variations within the 3 species causing heart rot in relation to their bearing on the taxonomy of the genus *Phytophthora*. The recorded host range of the heart-rotting species is extended and attention is called to the relationship between the disease of plants other than pineapple to the introduction and survival of heart-rotting species in pineapple fields.

The fungicidal control of heart rot has already been reported by the writer (11). Other papers will report epiphytological studies of heart rot and root failure of the pineapple plant caused by the same organisms.

Heart rot of the pineapple plant, *Ananas comosus* (Linn.) Merr., has been reported from Costa Rica (7), Jamaica (1), Puerto Rico (6), Cuba (4), Hawaii (11, 20), and Queensland, Australia (23). Its occurrence in Haiti and the Philippine Islands has been reported verbally to the writer by pineapple growers from these regions.

The rot is firm, cheese-like, and white, bordered by a characteristic brown margin (11). It destroys the younger regions of the stem and the basal portion of the central leaves.

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The Heart-rotting Organisms. The organisms reported to cause heart rot have been different species, mostly in the genus *Phytophthora*. Ashby (1) found *P. parasitica* Dastur causing heart rot of the Ripley variety in Jamaica. Bruner (4) stated that *P. terrestris* Sherb. caused a loss of 2 per cent in a planting of the Sugar-loaf variety in Cuba. Simmonds (23) consistently isolated an undetermined species of *Phytophthora* from heart-rot lesions of pineapple plants in Queensland.² Johnson (7) states that a bacterium was isolated and determined as the cause of the disease in Costa Rica.

In Hawaii, Sideris and Paxton (20) reported *Phytophthora meadii* McRae, *Phytophthora melongenae* Sawada, and *Pseudopythium phytophthoron* to be causal organisms. *P. meadii* was said to be the most widely distributed of the three.

Recent studies of the genus *Phytophthora* have called attention to the essential similarity of *P. parasitica* (2, 5, 9, 21) and *P. melongenae* Sawada. Preliminary studies by the writer demonstrated the practical impossibility of separating cultures from diseased pineapple plants into *P. melongenae* on the one hand and *P. parasitica* on the other, using morphology and size of zoosporangia as criteria. It seems more desirable to consider all such isolations as a single species, which, on the basis of precedent (9, 12), is herein designated *P. parasitica* Dastur. emend Ashby. Hence, pineapple-rotting forms classified by Sideris (19) as *P. melongenae* are treated in this paper as *P. parasitica*.

A close relationship has been shown by Leonian (9) and Tucker (21) to exist between *Phytophthora palmivora* (Butl.) Butl. and *P. meadii*. So closely are the species alike that Tucker has combined them as *P. palmivora*. Leonian merged both with *P. melongenae*, *P. parasitica* and others under the name of *P. omnivora* de Bary (9).

The pineapple strains of *Phytophthora* classified by Sideris as *P. meadii* and *P. melongenae* may be separated consistently, using Tucker's key (21), as *P. palmivora* and *P. parasitica*, respectively. Therefore, in consideration of Ashby's criticism (2) of *P. omnivora* as set up by Leonian (9), the designation *P. palmivora* will be used for the heart-rotting strains classified as *P. meadii* by Sideris and for the one similar isolation by the writer.

To learn the relative importance of the several *Phytophthora* species in Hawaii, isolations, in the present study, were made during a period of 3 years from diseased plants occurring in a large number of representative locations on the islands of Lanai, Molokai, Maui, Oahu, and Kauai. None of the several bacteria isolated were capable of reproducing the disease. Contrary to Sideris' findings, *P. parasitica* was found to be the most widespread of the pathogens. It occurred exclusively in all of the areas sampled

² Determined to be *P. cinnamomi* Rands by S. F. Ashby. Reported by Ashby in a letter to the writer.

on the islands of Lanai, Molokai, Maui, and Kauai. *P. palmivora* (*P. meadii*) was found in one field only, near Pupukea, Oahu. *P. cinnamomi*, found in one field on Oahu by Sideris and reported as *Pseudopythium phytophthoron* (19), has been found in pineapple fields from Moanalua to Waimea and Opaepa, a distance of approximately 30 miles on the western slopes of the Koolau range of mountains on Oahu. It is not in pineapple fields alone, however, being found abundantly at Kipapa in virgin soil at higher elevations than fruit culture. Here it was associated with dying plants of the fern *Dicranopteris emarginata*. It has not been found on any of the other islands, although a consistent search has been made for it.

PATHOGENICITY STUDIES

Heart Rot. A number of *Phytophthora* species from sources other than pineapple were tested in the laboratory to determine if they might cause heart rot. A standard technique was devised for these studies. A hole 5 mm. in diameter and 1 cm. deep was cut in the base of the stem of mature, cured crowns using a sterile cork borer. Into the hole were thrust malt-agar circlelets bearing the appropriate organism in pure culture. Incubation was in moist chambers, fashioned from Mason jars, at 25–27° C. for 5 days.

Two distinct groups were apparent among the organisms tested: (1) those listed in table 1 that caused heart rot, and (2) those listed in table 2 that caused no disease of pineapples. The separation of strains within single species is seen to be as follows:

Phytophthora cambivora, 6 isolations negative; *P. cinnamomi*, 5 positive, 3 negative; *P. palmivora*, 3 positive, 8 negative; *P. palmivora* (*P. arecae*), 2 positive, 1 negative; *P. palmivora* (*P. faberi*), 1 positive, 2 negative; *P. palmivora* (*P. meadii*), 2 positive; *P. parasitica*, 6 positive; *P. parasitica* (*P. melongenae*), 2 positive.

Among the cultures of *Phytophthora cinnamomi* studied, there is reason to believe that the strains originally isolated by Rands and designated 13.1, 31 and 35.1 have been handled in parallel, and have received similar treatment throughout their histories. Nevertheless, the respective abilities of these to infect the pineapple plant consistently separate them. So consistent are these differences that they appear to be of the magnitude recognized in the rust fungi as *physiological strains*. Indeed, in respect to this phase of their physiology, there is a greater difference between the strains of a single species than between certain strains of different species.

It has been demonstrated that similar differences in ability to produce disease are apparent when other species and other hosts are used (8, 21). That is, within a species there are strains that cause a specific disease and others that do not. The validity of separating any species of *Phytophthora* from another on the basis of the differential susceptibility of a single host,

TABLE 1.—*Strains of Phytophthora species that cause heart rot symptoms in wound-inoculated crowns of pineapple*

Designation of organism	Virulence	Source		Number ^a on culture
		Host	Author	
<i>P. cinnamomi</i>	+		Leonian
<i>P. cinnamomi</i>	+	Cinnamon	Rands to Drechsler	13.1 M
<i>P. cinnamomi</i>	+++	Cinnamon	Rands to Drechsler	35.1 B
<i>P. cinnamomi</i>	+	Rhododendron	White to Drechsler	x
<i>P. cinnamomi</i>	+++	Pineapple	Simmonds to Ashby
<i>P. palmivora</i> (<i>P. arecae</i>)...	++		Gadd to Baarn, Baarn to Leonian	1
<i>P. palmivora</i> (<i>P. arecae</i>)...	++		Gadd to Baarn, Baarn to Tucker	54
<i>P. palmivora</i> (<i>P. faberi</i>)...	++	Coconut in Philippines	Hartley to Leonian	75
<i>P. palmivora</i> (<i>P. meadii</i>)	+++	Hevea brasiliensis	Baarn to Sideris	55
<i>P. palmivora</i> (<i>P. meadii</i>)	+++	Pineapple	Sideris	54
<i>P. palmivora</i>	++	Coconut in Porto Rico	Tucker to Leonian	79
<i>P. palmivora</i>	++	Hevea brasiliensis	Leonian	82
<i>P. palmivora</i> "Cacao group"	++		Tucker from Baarn; Ashby to Baarn	57
<i>P. parasitica</i> (<i>P. melon- genae</i>)	+++	Eggplant	Baarn to Sideris	51
<i>P. parasitica</i> (<i>P. melon- genae</i>)	+++	Antirrhinum	Sideris	52
<i>P. parasitica</i> -Schwartz' strain	+++		Baarn to Sideris	44
<i>P. parasitica</i> -Dastur's strain	+++		Baarn to Sideris	45
<i>P. parasitica</i>	+++	Potato in Oklahoma	Drechsler to Tucker	P 9
<i>P. parasitica</i>	+++	Potato in Kentucky	Drechsler to Tucker	P10
<i>P. parasitica</i> (var. <i>Rhei</i> Leonian II)	++	Tomato	Nolla to Tucker	P19
		Rhubarb	Baarn to Tucker

^a These numbers were on the cultures when received and are presumably those of the original author whose name is given opposite the number in each case.

therefore, seems very questionable. Specifically, the validity of separating the species *P. cinnamomi* and *P. cambivora* (Petri) Buis, on the basis of the differential susceptibility of potato tubers to their attack is questioned. Such a separation has been made by Tucker (21).

In the present study susceptible pineapple crowns were inoculated with 6 separate isolations of *Phytophthora cambivora* and all gave negative results. However, 3 isolations of *P. cinnamomi* also were negative and would have been classified as *P. cambivora* if pineapple rather than potato had been the criterion of species separation.

TABLE 2.—Strains of *Phytophthora* species that do not cause heart rot symptoms in wound-inoculated crowns of pineapple

Designation of organism	Source		Number on culture ^a
	Host	Author	
<i>P. cactorum</i> (<i>P. fagi</i>)	Beech	Baarn to Sideris	41
<i>P. cactorum</i>		Baarn to Sideris	57
<i>P. cambivora</i>		Drechsler	—
<i>P. cambivora</i>	Chestnut in Secondigny, France	J. Dufrenoy to Ashby	—
<i>P. cambivora</i>	Chestnut in England	W. R. Day to Ashby	—
<i>P. cambivora</i>		Petri to Baarn	
<i>P. cambivora</i>		Baarn to Leonian	4
<i>P. cambivora</i>		Same as above	5
<i>P. cambivora</i>		Petri to Baarn	
<i>P. cinnamomi</i>	Cinnamon	Baarn to Tucker	53
<i>P. cinnamomi</i>	Chestnut in S. Europe	Rands to Drechsler	31
<i>P. cinnamomi</i>	Walnut in Australia	Petri to H. Fawcett	Fawcett's
<i>P. cinnamomi</i>		Fawcett to Ashby	208
<i>P. cinnamomi</i>		Ashby	—
<i>P. citrophthora</i>		Baarn to Sideris	47
<i>P. hydrophila</i> ^b		Baarn to Sideris	48
<i>P. infestans</i>	Potato	Baarn to Sideris	49
<i>P. palmivora</i> (<i>P. arecae</i>)		Leonian	2
<i>P. palmivora</i> (<i>P. faberi</i>)		Leonian	76
<i>P. palmivora</i> (<i>P. faberi</i>)		Leonian	77
<i>P. palmivora</i> ^c "Cacao group"	Papaya	Sideris	43
<i>P. palmivora</i> ^c "Cacao group"	Ricinus	Sideris	53
<i>P. palmivora</i>	Borassus in India	Hartley to Leonian	80
<i>P. palmivora</i>		Baarn to Leonian	78
<i>P. palmivora</i>	Grapefruit in Puerto Rico	Tucker to Leonian	81
<i>P. palmivora</i>		Ashby to Baarn	
<i>P. palmivora</i>		Baarn to Leonian	83
<i>P. palmivora</i> "Rubber group"	Coconut in Mauritius	Ashby	—
<i>P. palmivora</i> "Rubber group"	Coconut in Puerto Rico	Tucker	55
<i>P. palmivora</i> "Cacao group"	Coconut in S. India	Ashby	—
<i>P. palmivora</i> "Cacao group"	Palmyra palm in India	Ashby	—
<i>P. parasitica</i> var. <i>nicotiana</i>	Tobacco	Baarn to Sideris	—
<i>P. richardiae</i>		Baarn to Sideris	42
<i>P. parasitica</i> (<i>P. terrestris</i>)		Baarn to Sideris	56
<i>P. sp.</i> ^d	<i>Ficus carica</i>	Sideris	58

^a These numbers were on the cultures when received and are presumably those of the original author whose name is given opposite the number in each case.

^b This species is included in *P. omnivora* de Bary by Leonian and in *P. capsici* Leonian by Tucker.

^c Subcultures of these two organisms were distributed by Sideris under the name *P. monoana* n. sp. but a description was not published. S. F. Ashby, in a letter to the writer, stated that number 43 is a typical strain of *P. palmivora* in the "Cacao group" of that species. C. M. Tucker, also, independently, made a similar designation.

^d This culture was received from Sideris bearing the name *P. symmetrica* n. sp. A description of it has not been published and its identity has not been determined.

The writer has shown that bud rot of the palm and heart rot of the pineapple plant may be caused by the same strains of *Phytophthora* (Table 1, organisms numbered 1, 54, 55, 75, 79). Since this is true it is regarded as significant that both diseases occur in proximity on at least 2 of the islands of the Hawaiian group. For a comparison of these 2 diseases, see the references cited as numbers 11, 18 at the end of this paper.

It also has been demonstrated that heart rot of the pineapple plant is caused by the same pathogens that cause: (1) foliage and fruit rot of the rubber tree, *Hevea brasiliensis*; (2) cankers of the cinnamon tree, *Cinnamomum burmanni*; (3) canker of *Rhododendron* sp.; (4) foliage and stem blight of the tomato, *Lycopersicon esculentum*; (5) leaf blight and tuber rot of the potato, *Solanum tuberosum*; and (6) crown rot of rhubarb, *Rheum rhaponticum*.

This suggests the means by which the heart-rotting organisms may have been introduced into the islands. It also indicates the desirability of restricting the growth of certain vegetables where the pineapple disease is prevalent.

Green-fruit rot:

During February, 1932, pineapple fruits of four stages of development were inoculated with local isolations of *Phytophthora cinnamomi*: A, inflorescences on which the last flowers had just withered; B, half-grown green fruits; C, full-grown green fruits; and D, yellow ripe fruits. An agar circlet containing the organism was placed over needle punctures made in a single eye, or on the cut stem base. *P. cinnamomi* aggressively rotted every inflorescence, rapidly extending the lesions. Half-grown fruits also were invaded, but the rate of advance of the fungus was slow. No full-grown fruit, either green or yellow, rotted in the several tests. All tissues of the invaded fruits appeared susceptible, the core rotting as well as other parts.

A confirmatory test was conducted during February, 1934. Fruits corresponding to the first 3 classes in the earlier test were selected in pairs, each pair being nearly uniform in weight and appearance. One fruit of each pair was inoculated with culture 60 of *Phytophthora cinnamomi* by placing an agar culture in a wound made with a 5 mm. cork borer. The crowns and peduncles were then removed from the other fruits. They were cut so that separate determinations of the pH of juice, expressed by hand, could be made on the shell, the core, and the upper and lower halves of the flesh. Table 3 shows the weights and pH of these fruits. Following an incubation of 15 days at 25–27° C. the inoculated fruit of each pair was examined. All inoculations made in inflorescences of class A were positive regardless of their position on the fruit. All fruits of class B likewise showed disease; inoculations had been made in the upper and lower halves

TABLE 3.—*The weight and hydrogen-ion concentration of immature fruits inoculated with Phytophthora cinnamomi*

Maturity of fruit ^a	Hydrogen-ion concentration of fruit parts ^b											
	Weight of fruit in grams		Flesh						Core		Shell	
			Upper half		Lower half							
Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.			
A	450- 520	485	4.72-5.08 ^c	4.96								
B	1380-1580	1458	3.79-4.00	3.87	3.48-3.94	3.75	3.59-4.23	3.98	4.21-4.64	4.49		
C	2640-2960	2770	2.75-3.29	2.94	2.41-2.99	2.66	2.75-3.01	2.91	3.58-3.71	3.65		

^a The fruit classes are as follows: A, inflorescences with last flowers just withered; B, half grown green fruits; C, fully grown green fruits.

^b Determinations made with hydrogen electrode.

^c All parts of the inflorescences of class A were composited.

of the fruit as well as in the core at the base of the fruit. In all cases lesions from inoculations in the upper regions of the fruit expanded more rapidly than similar inoculations in the lower regions.

No inoculations in any fruit of class C produced a lesion, thus verifying the earlier observations. Figure 1 shows a typical fruit of class B. Observe the difference in size of the lesions, and the distribution of disease through all of the tissues of the fruit, including the core and the shell. Note the characteristic brown margin bordering the grayish white, firm, rotted areas.

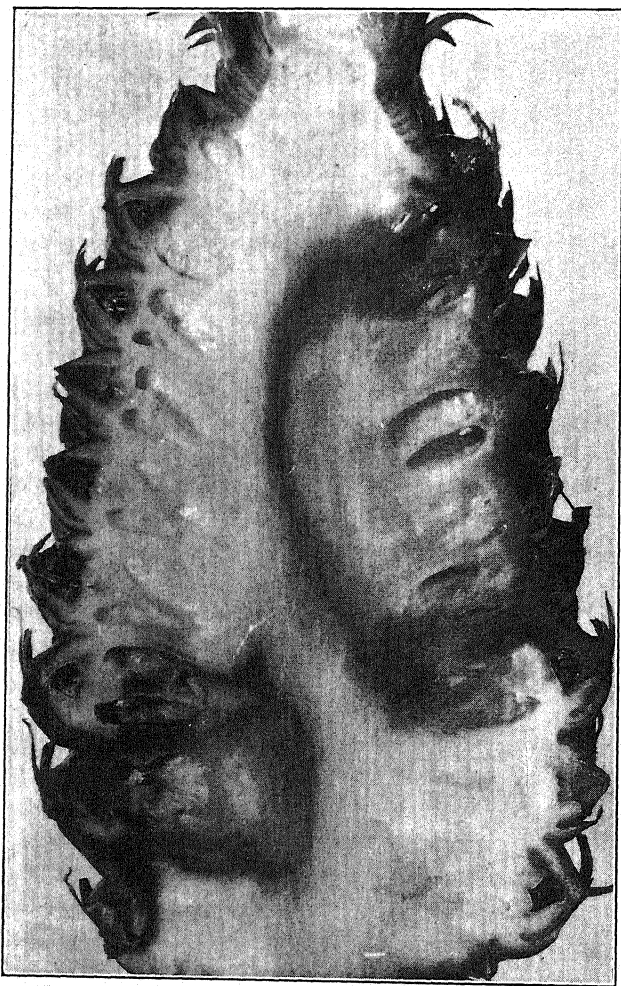


FIG. 1. Rot of green pineapple fruit caused by *Phytophthora cinnamomi*. Photographed 15 days after inoculation. Note the dull white, dry rotted areas surrounded by the characteristic brown margins. Observe that the basally situated lesion is the smaller one.

A difference also is noticeable in the color of the healthy and diseased shell areas: the infected areas are brownish and may be covered with white mycelium. In one case this mycelium had grown over the superficial tissues of the shell to the crown of the fruit, where typical heart rot developed. The lesion, however, had not expanded so rapidly through the internal tissues.

Similar results were obtained with *Phytophthora parasitica* (*P. melongenae*), but no disease was produced by *P. palmivora* (*P. meadii*). Young inflorescences of a series of fruits susceptible to the other species under similar conditions, were repeatedly inoculated with *P. meadii* by both the writer and C. H. Spiegelberg,³ but disease developed in no single instance.

Waldron, in 1928, first described a rot of green pineapple fruits occurring naturally on the islands of Kauai and Oahu. *Phytophthora meadii* was reported⁴ to be the pathogen and disease was reported to have been experimentally reproduced using this organism. The symptoms she described are similar to those illustrated above in figure 1, produced in the present study with *P. cinnamomi* and *P. parasitica* only.

No disease was found in the field from 1928 until January, 1933, when a single specimen was found on Kauai by M. B. Linford. It is possible that green fruit rot caused by the heart-rotting *Phytophthoras* may again cause significant losses.

Waldron⁴ reported: "Susceptibility to *Phytophthora* rot appears to depend on the lower acidity of younger fruits, which have pH values ranging between 5.4 to 4.4, whereas those of fully developed fruits, which are resistant to the disease, lie between 4.4 and 3.4."

The results of the present study seem to substantiate a correlation between pH and development of the disease: Not only did disease fail to develop in the more acid fruits, but, in fruits where lesions were evident, the disease had extended most rapidly through the tissues of lowest acidity.

Weed and Green-manure Plants.—As a phase of epiphytological studies carried on during a period of 3 years, a number of weeds common in cultivated and fallow pineapple fields were tested. Also the commoner green-manure plants were inoculated. Table 4 summarizes the results.

The ability of these *Phytophthoras* to infect wounded weed or green-manure plants, while perhaps of little taxonomic significance (22), is nevertheless, indicative of material suitable for the growth of the heart-rotting organisms in the absence of the pineapple, and thus constitutes a factor in survival of the pathogenes in the field. Plowing and disking periodically produce such wounded materials in abundance, often during weather suitable for growth of these fungi. From this standpoint it is significant that the 3 common green manure plants, *Crotalaria*, *Mucuna*, and *Cajanus*, are

³ Unpublished notes, 1930.

⁴ Private communication to the local pineapple industry, 1928 and 1930.

TABLE 4.—Weeds and green-manure plants tested for susceptibility to pineapple heart-rotting organisms

Plant tested and part used	Organisms tested and inoculation type											
	44 ^a		52		54		60		384		443	
	W	NW	W	NW	W	NW	W	NW	W	NW	W	NW ^b
<i>Carica papaya</i> L. seedlings	+	-	-	-	+	-	-	-	-	-	-	-
<i>Crotalaria incana</i> L. seedlings	+	+	+	-	+	+	-	-	-	-	-	-
<i>Macuna gigantea</i> (Willd.) DC. seedlings	+	-	+	-	+	-	Sl. +	-	-	-	-	-
<i>Cajanus cajan</i> (L.) Millsp. seedlings	+	-	+	-	+	+	+	-	+	-	-	-
<i>Lycopersicon esculentum</i> Mill. seedlings	+	+	+	-	+	-	Sl. +	-	Sl. +	-	Sl. +	-
<i>Melia azedarach</i> L. seedlings	-	-	-	-	-	-	-	-	-	-	-	-
<i>Melia azedarach</i> L. roots	-	-	-	-	-	-	-	-	-	-	-	-
<i>Melia azedarach</i> green fruits	-	-	+	-	-	-	-	-	-	-	-	-
<i>Ricinus communis</i> L. seedlings	+	+	Sl. +	-	Sl. +	-	-	-	-	-	-	-
<i>Ricinus communis</i> L. radicles	-	-	+	+	+	+	+	+	-	-	-	-
<i>Emelia sonchifolia</i> DC. seedlings	-	-	-	-	-	-	-	-	-	-	-	-
<i>Richardsonia scabra</i> St. Hil. seedlings	+	+	+	+	+	-	- to	-	-	-	+	-
<i>Agrotum conyzoides</i> L. seedlings	-	-	-	-	-	-	Sl. +	-	-	-	-	-
<i>Bidens pilosa</i> L. seedlings	+	Sl. +	-	-	-	-	-	-	-	-	-	-
<i>Erigeron canadensis</i> seedlings	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dicranopteris emarginata</i> (Brack) W. J. Robinson, young fronds	-	-	-	-	-	-	-	-	-	-	-	-

^a The numbers refer to Phytophthora species: 44—*P. parasitica*; 52—*P. parasitica* (*P. melongenae*); 54—*P. palmivora* (*P. meadii*); 60, 384, 443—Hawaiian isolations of *P. cinnamomi*; 303—*P. cinnamomi* from Leonian.

^b W indicates inoculation made following wounding of host; NW indicates no wounding.

susceptible to more than one of the heart-rotting species. Two of the widely distributed weeds, *Ricinus* and *Richardsonia*, are likewise very susceptible, giving a basis for the observation that where *Ricinus* occurs in abundance, heart rot also occurs.

Economic Vegetable and Flowering Plants. In an effort to prove the synonymy of *Phytophthora cinnamomi* Rands and *Pseudopythium phytophthoron* Sideris, the pathogenicities of authentic strains of *Phytophthora cinnamomi* were compared with Sideris' organism. Plant parts used by Tucker in his studies were inoculated with 3 isolations of *Pseudopythium* and with 3 isolations that served as the type for Rands' description of *P. cinnamomi*.

The pathogenicities of these 6 cultures are compared in Table 5 with Tucker's cultures 242 and 244 of *Phytophthora cinnamomi* and a single culture of *P. cambivora*. The 3 isolations of *Pseudopythium* all agree favorably with the strains of *Phytophthora cinnamomi*. Two of Rands' original isolations cause heart rot of pineapple crowns. One of these, 35.1, compares favorably in virulence with Hawaiian strains from pineapple. Other strains obtained from Leonian and Drechsler are slightly pathogenic (Table 1).

The finding of *Phytophthora cinnamomi* as a cause of heart rot in Queensland, Australia, (isolations of Simmonds identified by S. F. Ashby) strengthens the prior conclusion by the writer that *Pseudopythium* is synonymous with this species (10). To confirm additionally the identity of *Phytophthora cinnamomi* and *Pseudopythium*, 5 local isolations from pineapple were sent to R. P. White for comparison with his isolations from *Rhododendron*.⁵ All were pathogenic on *Rhododendron* and agreed as to type of canker produced on the stems and as to rate of invasion. White's cultures from *Rhododendron* were found to be only mildly pathogenic on pineapple, as shown in table 1.

CULTURAL STUDIES

Physiology. Tucker, who studied the effect of temperature on the rate of growth of a number of *Phytophthora* species, found that 2 strains of *P. cinnamomi* failed to develop above 35° C. while 3 other isolations failed to develop above 32.5° C. The optimum temperature for growth he reported lies between 20° and 25° C.

Table 6 presents the results of similar studies made with a subculture of the type of Sideris' *Pseudopythium*, organism 60. Petri dish plates (100 mm.) containing 12 cc. of Difco corn meal agar (pH 6.4) were inoculated with 5 mm. discs of similar medium cut from the marginal growth of a 72-hour culture. These were incubated in triplicate at the several

⁵ Results reported by letter to the writer, April 30, 1932.

TABLE 5.—A comparison of the pathogenicities of cultures of *Pseudopythium phytophthoron* Sideris with *Phytophthora cinnamomi* Rands and *Phytophthora cambivora*^a

Host plant and part used	Organisms used and pathogenicity									
	Pseudopythium phytophthoron			Phytophthora cinnamomi						
	60	384	443	13.1	31	35.1	242	244	Phytophthora cambivora	
Papaya seedlings	-	-	-to V. sl. +	(-)	(-)	(-)	(-)	(-)	-	-
Apple fruits	+	+	+	(+)	(+)	(+)	(+)	(+)	+	+
Potato tuber	++	++	++	(++)	(+)	(+)	(++)	(++)	-	-
Tomato fruits	+	+	+	(+)	(-)	(+)	(+)	(+)	-	-
Egg plant fruits	-	-	-	(-)	(-)	(-)	(-)	(-)	-	-
Ricinus seedlings	-	-	-	(-)	(-)	(-)	(-)	(-)	-	-
Tomato seedlings	-	-	-	(-)	(-)	(-)	(-)	(-)	-	-
Egg plant seedlings	-	-	-	(-)	(-)	(-)	(-)	(-)	-	-
Bryophyllum leaves ..	-	-	-	(-)	(-)	(-)	(-)	(-)	-	-
Pineapple crowns	++	++	++	+	-	+	(-)	(-)	-	-

^a Cultures numbered 60, 384, 443 are local isolations from pineapple. Cultures 13.1, 31, and 35.1 are strains of *P. cinnamomi* isolated by Rands from *Cinnamomum burmanni* in Sumatra. Cultures 242 and 244 are Tucker's isolations from *Persea persae* in Puerto Rico. The data in parenthesis are taken from Tucker's paper (21) but were verified for organisms 13.1, 31, 35.1.

TABLE 6.—*The mean diameter of mycelial growth in plate cultures of Phytophthora cinnamomi (Pseudopythium phytophthoron) strain 60 on cornmeal agar (pH 6.4) incubated 96 hours at various temperatures*

Temperature degree C.	Mean diameter in millimeters	
	Range of 3 plates	Average
12.8 ± 0.5	4.5 - 4.5	4.5
15.6 ± 0.5	17.5 - 23.0	20.1
18.3 ± 0.5	27.5 - 27.5	27.5
21.1 ± 0.5	35.5 - 38.5	38.5
23.9 ± 0.5	36.5 - 43.5	40.0
25.0 ± 1.0	52.5 - 55.5	54.0
26.7 ± 0.5	44.5 - 44.5	44.5
27.8 ± 0.5	33.5 - 34.5	34.0
30.0 ± 1.0	34.5 - 39.5	36.5
32.5 ± 1.0		No growth ^a
35.0 ± 1.0		" "
37.5 ± 1.0		" "
40.0 ± 1.0		" "

^a Slight growth less than 1 mm. occurred in one plate.

temperatures for 96 hours, and then the diameter of growth was determined (colony diameter minus 5 mm.). The fluctuation between plates, as well as the average for each series, is given. The optimum temperature for growth is 25° C.; the maximum is 30° C.; the minimum near 10° C. The cardinal temperatures of other local isolations, 384, 407, and 443, are identical with those reported for strain 60. Hence, local isolations compare favorably in temperature relationships with Rands' organism 35.1, reported as number 174 in Tucker's paper (21).

While the cardinal temperatures for the several strains of *P. cinnamomi*

TABLE 7.—*The mean diameter of mycelial growth of strains of P. cinnamomi grown on malt agar for 72 hours*

Strain ^a	Mean colony diameter mm. ^b		
	25° C.	30° C.	35° C.
443	49.0	49.5	0
35.1	49.5	59.0	0
Leonian's	63.0	42.3	0
13.1	38.5	39.0	0

^a For source of cultures see table 1.

^b The mean value shown is for five plates at each temperature.

TABLE 8.—The mean diameter of mycelial growth of strains of heart rotting *Phytophthora* sp. grown on papaya agar for 72 hours at 25–27° C.

Organism	Strain	Source	Mean colony diameter mm. ^a
<i>P. cinnamomi</i>	60	Waialua, Oahu	66.0
“	407	Helemano, Oahu	60.5
<i>P. palmivora</i>	54	C.B.S. Baarn	54.0
“	55	“ “	43.3
<i>P. parasitica</i>	417	Pupukea, Oahu	38.2
“	415	Hanapepe, Kauai	37.3
“	414	Kualapuu, Molokai	34.5
“	401	Kipu, Molokai	32.5
“	393	Island of Lanai	28.5
“	51	C.B.S. Baarn	25.8

^a The mean value shown is for 5 plates of each organism.

appear to be very similar, their rates of growth are dissimilar throughout a range of temperatures. As an example, contrast the colony diameters of strains 35.1 and 13.1 shown in table 7. It is important to note, moreover, that the rate of growth of these organisms on artificial media is not necessarily related to their virulence, shown in table 1. Leonian's strain and strain 13.1 advance at about an equal rate through pineapple tissue, but their rates of growth on agar are very different (Table 7).

Similar differences occur between the 3 species that cause heart rot: *P. cinnamomi*, *P. palmivora*, and *P. parasitica*. Papaya (20) and malt

TABLE 9.—The mean diameter of mycelial growth of *P. palmivora* and *P. parasitica* in plate cultures on corn-meal agar (pH 6.4) incubated 96 hours at various temperatures

Temperature degrees Centigrade	Mean colony diameter in mm. ^a	
	<i>P. palmivora</i> Strain 55	<i>P. parasitica</i> Strain 414
12.8 ± 0.5	30.5	4.0
15.6 ± 0.5	41.0	22.0
18.3 ± 0.5	58.0	32.0
21.1 ± 0.5	66.0	38.0
23.9 ± 0.5	66.0	43.7
26.7 ± 0.5	63.2	46.0
30.0 ± 1.0	43.0
32.5 ± 1.0	25.0	40.0
35.0 ± 1.0	0	20.0
37.5 ± 1.0	0	8.0
40.0 ± 1.0	0	0

^a The mean value shown is for 5 plates at each temperature.

agars (12) seem to accentuate differences in rate of growth of the 3 species, as well as the strains of a single species, as shown in table 8. On corn-meal agar the differences are consistently in the same direction, but of a lesser magnitude. No similar differences have been noted in the rate at which heart rot lesions are extended. These observations suggest differences in the enzyme activities of the several strains.

Table 9 shows the rates of growth of typical strains of *Phytophthora palmivora* and *P. parasitica* at various temperatures. These growth curves should be compared with that of *P. cinnamomi* shown in table 6. *P. cinnamomi* is distributed in the cooler upland areas of Oahu, only, while *P. parasitica* is found also in the hotter locations, near the sea level on 3 of the islands sampled. It apparently is more tolerant of higher temperatures.

Table 9 shows that no growth of strain 55 of *Phytophthora palmivora* occurred at 35° C., but that strain 414 of *P. parasitica* did grow at this temperature. This difference is in agreement with the observations of Tucker who regards it to be of diagnostic value in separating the two species (21). For the large number of strains of both species with which Tucker worked this temperature relationship appeared to be constant, but for isolations of *P. parasitica* from pineapple plants it does not seem to hold. Table 10 is a summary of the behavior of a number of representative isolates from

TABLE 10.—*The behavior of heart rotting strains of Phytophthora incubated in plate cultures of Difco corn-meal agar (pH 6.4) for 4 days at 35° C.*

Organism number	Source	Growth at 35° C. ^a	Growth at 25° C. following incubation at 35° C. ^b
72	Heleman, Oahu	—	+
77	Kilauea, Kauai	—	—
395	Maunaloa, Molokai	—	+
398	Brodie, Oahu	—	—
399	Heleman, Oahu	—	+
400	Kualapuu, Molokai	—	+
401	Kipu, Molokai	+	+
417	Pupukea, Oahu	+	+
AC 34	Miki, Lanai	+	+
414	Kipu, Molokai	+	+

^a The sign + indicates growth, the sign — indicates no growth.

^b Incubation at 25° C. followed incubation of 4 days at 35° C.

heart-rot lesions. If growth at 35° C. on corn-meal agar were the criterion for separating the species, the first 6 isolates would be classified as *P. palmivora* and the last 4 as *P. parasitica*. Morphologically, however, all of the organisms appear to be strains of *P. parasitica* Dast. emend Ashby. The sporangia, chlamydospores, and mycelial characters more nearly agree with

typical strains of this species than with *P. palmivora*. Hence, the growth or lack of growth of *Phytophthora* strains at 35° C. on corn-meal agar seems an unsuitable basis for separating *P. palmivora* and *P. parasitica*.

Morphology. A comparative examination of the morphology of Hawaiian isolations 60, 384, and 443 of the organism named *Pseudopythium phytophthoron* by Sideris with strains 31 and 35.1 of *Phytophthora cinnamomi* Rands isolated by Rands revealed no significant differences in the

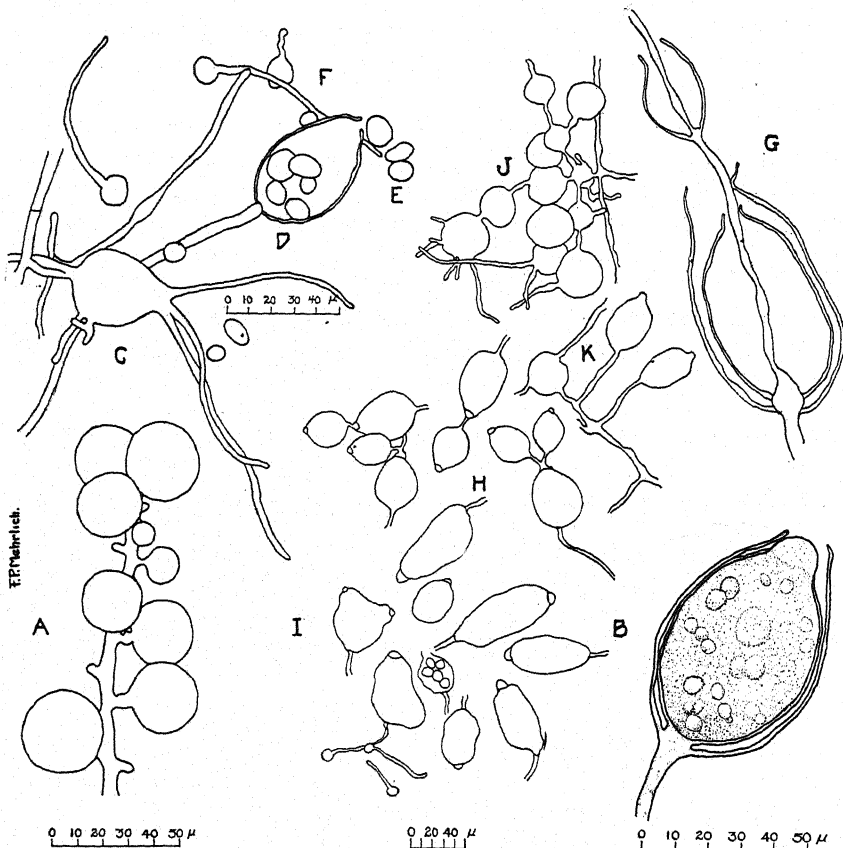


FIG. 2. Asexual reproductive structures of Hawaiian isolations of *Phytophthora cinnamomi* Rands (*Pseudopythium phytophthoron* Sideris) drawn with camera lucida. A. Cluster of chlamydospores. B. Full-grown papillate, primary sporangium within the empty walls of a dehiscent sporangium. C. Primary sporangium germinating by germ tubes. D. Dehiscent sporangium containing encysted zoospores. E. Zoospores shortly after being discharged from sporangium. F. Zoospores germinating by vegetative germ tubes. G. Sporangia showing proliferation. H. Sporangia germinating by the production of secondary sporangia. I. Primary sporangia of various shapes. J. Hyphal vesicles resembling chlamydospores produced in M/100 KNO₃. K. Hypha bearing both sporangium and a vesicle.

size of mycelium, "chlamydo-spores," hyphal vesicles, or conidia when grown under similar conditions. The diameter of hyphae of the Hawaiian isolations average 10 microns; (on Difco corn-meal agar, pH 6.4). Lateral branches are only slightly smaller, rarely being less than 5 microns.

The "chlamydo-spores" of the Hawaiian isolations may be wholly spherical, varying in diameter from 10 to 36 μ , averaging 20 μ at the bottom of Petri plate cultures and 25 μ on aerial mycelia. They may intergrade in both form and dimensions with the abundant hyphal vesicles of irregular shape and size, or may occur in grape-like clusters, or be disposed singly and terminally on short lateral branches (Fig. 2). The "chlamydo-spores" germinate by germ tubes, 1 to 6 or more. The germ tubes in liquid cultures may give rise to vegetative hyphae or rarely produce zoosporangia. The ellipsoidal zoosporangia are produced in very dilute liquid media only, borne on short (50–75 μ) or long (75–790 μ) simple or branched sporangiophores. They are irregular in size, shape, and proportions and are characterized by successive proliferation. The methods to induce zoospore formation are reported elsewhere (13). Produced by those methods, the majority of the zoosporangia of Rands' cultures and the Hawaiian isolations show conspicuous papillae (Fig. 2). These contrast with the blunt, nonpapillate sporangia reported by all other workers. Of 225 sporangia counted in a nonsterile soil solution (pH 4.5), 183 were papillate. Distorted sporangia showing more than one papilla are not rare. Dehiscence occurs at the base of the papilla, the opening ordinarily being less than the diameter of the zoospores. Zoospores, although fully formed, may not be discharged. In such cases they germinate within the sporangium and send the germ tubes through its walls. Zoospores may not be formed at all, the conidia giving rise to one or more hyphae or to secondary conidia. Characteristically 2 secondary conidia are formed, although 1 to 3 have been observed to originate from a single primary conidium. The zoospores are active only a short time under conditions observed, germination at 20° C. taking place between 4 and 5 hours after the spores are liberated. Various of these stages are illustrated in figure 2, by camera-lucida drawings of local isolation 60 of *Phytophthora cinnamomi* (*Pseudopythium phytophthoron*).

In table 11 the extensive variability of zoosporangia is demonstrated by isolation 384 in a soil extract solution of approximately pH 4.5. The ratio of sporangial length to width varied between 1.33 and 2.22; average 1.77. Zoospores liberated either from the larger or smaller sporangia show a range of sizes, a few being either much larger or smaller than the average. The number of spores liberated is directly correlated with the size of the sporangium. Twenty-five zoospores selected at random from sporangia recorded in table 11 measured in microns after coming to rest: 3.8–10 \times 8.8–13.9, average 6.4 \times 11.6.

TABLE 11.—The size in microns of representative zoosporangia of *P. cinnamomi*, strain 884, produced in soil-extract solution pH 4.5

Length	Width	22.68	27.27	27.72	30.24	30.48	32.76	33.02	35.28	35.56	37.80	38.10	40.64	43.18	45.72	47.88	48.26	50.80	53.34	55.88	Total
47.88								1												2
49.14			1	1		1		1												1
50.40			1	1		1		1												4
50.80											1									1
52.92			1	1		2				1										3
55.44						1	1		1											2
55.88																				5
60.48		1						1	1											1
60.96						1						2								1
61.70								1												4
65.52	1								1			2	1	1						1
66.04												4	2							6
68.58									1				1							1
71.12					1								1	1						2
73.66													1			1				5
76.20															1					1
78.74						1														1
80.56																				1
80.72																				1
81.28																				12
86.36																				4
88.90																				1
91.44																		1		3
93.98																				1
96.52																				2
99.06																				1
101.60																				2
Total	1	1	1	4	2	9	1	3	3	1	3	18	9	7	2	7	3	1	3	73

Attempts to produce oospores have been unsuccessful, although media and temperatures used by others to produce oospores in other strains have been duplicated. Local isolations paired variously with other local isolations, as well as with Rands' strains 13.1, 35.1 and Simmonds' strain from pineapple in Australia, have all given negative results.⁶ Pairings also were made with several strains of *Ph. cambivora* but were likewise negative.

It is believed that the essential morphology of the local isolations 60, 384, and 443, described above, is sufficiently similar to authentic strains of *Phytophthora cinnamomi* Rands to indicate the synonymy of this species and *Pseudopythium phytophthoron* Sideris.

The 6 strains of *Phytophthora cambivora* (Petri) Buis. studied comparatively with 6 strains of *P. cinnamomi* showed a close similarity to *P. cinnamomi*. The differences between the species were very minor ones in amount of growth of aerial mycelium and in abundance of "chlamydospores" produced on agar plates or slants. These differences are suggestive of strain or, at best, of varietal differences of a single species, only.

Between the size and shape of zoosporangia or size and character of oogonia produced by the 2 species, there appear to be no significant differences recorded by other workers (2, 3, 9, 14, 15, 16, 17, 21) (Footnote 6, p. 15). Throughout the range of usual physiological reactions and over a considerable host range the 2 species are similar (21). It is suggested, therefore, that *P. cambivora* (Petri) Buis. and *P. cinnamomi* Rands be combined as a single species.

SUMMARY

The record of the world distribution of pineapple heart rot is extended and brought up-to-date. The results of a 3-year study on the distribution in the Hawaiian Islands of the 3 local causal organisms are given, indicating the presence of *Phytophthora parasitica* on all of the islands sampled, *P. cinnamomi* over a distance of 30 miles on one drainage slope of Oahu only, and *P. palmivora* in a limited area on Oahu, only. These findings are in contrast with the distribution reported for the species in the islands by Sideris and Paxton (20).

Synonymies of the heart-rotting pathogens are discussed, and detailed evidence is presented for considering *Pseudopythium phytophthoron* Sideris as a strain of *Phytophthora cinnamomi* Rands.

⁶ In a communication to the writer S. F. Ashby reported the following dimensions for the sexual organs of Simmonds' strain of *P. cinnamomi*, in microns: Oogonia (mean of 62) 45.4, range 37-58; oospores (mean of 19) 38.5, range 33-49; antheridia mostly 16-29 × 16-25, but occasionally transversely septate and elongate, up to 33 × 21. The oogonia were reddish brown; the antheridia amphigynous. Only one-third of the oogonia contained differentiated oospores. They were formed at the base of a Quaker oats slant after 45 days at a mean temperature of about 16° C.

Variations are demonstrated between cultures within the species *P. cinnamomi*, *P. palmivora*, and *P. parasitica*, of a nature recognized in the rust fungi as *physiological strains*. The recognition of these differences casts doubt on the validity of separating species of *Phytophthora* on the basis of the differential susceptibility of a single host.

The validity of separating *P. cinnamomi* Rands from *P. cambivora* (Petri) Buis. by the differential production of rot of potato tubers (21) is questioned. Since the morphology of the 2 species is essentially similar, it is suggested that they be combined as the single species *P. cambivora*, in which strain differences may be recognized.

Data are presented showing that a number of *P. parasitica* isolates from pineapple heart rot in Hawaii are more sensitive to high temperatures than strains studied by Tucker (21). Since these organisms do not grow on cornmeal agar at 35° C., this criterion of Tucker's for separating *P. parasitica* from *P. palmivora* does not seem reliable.

New host-range relationships of the pineapple pathogens are discussed, including an experimentally produced green pineapple fruit rot. The relationship of Rhododendron canker, bud rot of palms, foliage and fruit rot of the rubber tree, canker of the cinnamon tree, and rots of common weed, truck-crop and green-manure plants of pineapple fields to pineapple heart rot is reported.

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THE IDENTITY OF RASPBERRY MOSAICS¹

L. M. COOLEY

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Different opinions exist as to the number of viruses concerned in the etiology of mosaic diseases of raspberries in eastern North America. Because of this much confusion and uncertainty has ensued in the general understanding about these maladies.

While the writer was investigating the nature and identity of the so-called "mild mosaic" in the Columbian variety of the hybrid or purple raspberry (*Rubus neglectus* Peck), results appeared that had a direct bearing on the larger question of the number of raspberry mosaic viruses. It is hoped that presentation of this evidence and a discussion of the experiences and opinions other investigators have recorded in the literature will simplify and clarify concepts of the raspberry mosaics.

NATURE AND IDENTITY OF THE "MILD MOSAIC" IN COLUMBIAN RASPBERRIES

Survey of the Problem

The variety Columbian is the only purple raspberry of commercial importance, but it is grown extensively in some sections. Foliage of plants of this variety usually exhibits a mild mottling, accompanied by little or no injury. So common is the occurrence of this mottling in Columbian stock that it has come to be regarded by growers as a varietal characteristic. This mottling is usually most evident and general on the first leaves produced by fruit-spur growth in the spring. It also occurs noticeably on the first leaves of the new shoots (turions) and may become evident sporadically on foliage produced later. Leaves that are grown during or following cool weather especially are apt to show the mottled effect.

Such symptoms when found in black raspberries have been designated "mild mosaic" by Dodge and Wilcox (7) and by Bennett (1). However, opinions in literature as to the nature and relationships of this mild mottling in hybrid raspberries have been indefinite and variable and largely a matter of conjecture.

In 1927, Bennett (1) included in a discussion of red (raspberry) mosaic "two rather distinct types of mottling" occurring in Columbians. The milder of his two types is apparently identical with the "mild mosaic" of Columbian raspberries dealt with herein. But no statements of inoculation trials on indicator black raspberry plants were given to support placement of this disease as red (raspberry) mosaic. In later contributions,

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Bennett (2, 4) classified this mild mottling of purple raspberries with "mild mosaic" as affecting all species of raspberries.

Rankin's published comments reflect an evolution in ideas pertaining to this question. In 1924, he apparently did not recognize this mild mottling as a mosaic and stated (8, p. 1): "The purple variety Columbian is practically free of mosaic in the southwestern part of Erie county and in Chautauqua county [New York]." In 1927, he published these statements (9, p. 9): "'Mild mosaic' of raspberries is of doubtful nature and of little or no commercial importance, although widely prevalent. . . . It is suspected that 'mild mosaic' is due to a temporary infestation of red mites which cause this injury of feeding on the unfolding leaves. . . . Many standard purple varieties seem to be more favorable hosts for the rapid multiplication of the red mite and the typical injury is common on such varieties." In March, 1931, (11, p. 5, 6) he indicated a suspicion that the "mild mosaic" of Columbians may be caused by the red (raspberry)-mosaic virus in the following assertions: "It seems doubtful if there exists a distinct virus disease that should be called mild mosaic. This statement includes the so-called 'mild mosaic' in purple varieties in which it is believed that all types of virus mottling are referable to either red (raspberry) mosaic or to yellow mosaic."

Cooley and Rankin (6, p. 6), in December, 1931, reported that, "In some of the experimental plantings the source of red [raspberry] mosaic invasion seemed to be traceable to nearby cultivated Columbian plantings, showing the 'mild mosaic' typical of this variety." These findings of field study were considered proof that the mild mosaic in purple raspberries was identical with red (raspberry)-mosaic in black raspberries. In 1933, Rankin (13, p. 30) stated that, "All Columbian stock has been uniformly affected with red [raspberry] mosaic for several years."

It is singular that no experimental inoculation tests made for the specific purpose of determining the nature and identity of this malady have been reported. Bennett (5), in studies on the constancy of degree of raspberry-mosaic symptoms, reported that 11 of 25 healthy black-raspberry plants (Cumberland variety) became affected with *mild mottling only* when aphids of the species *Amhorophora rubi* were transferred to them from mildly mottled Columbians. This appears inconsistent with his classification in 1927 of the mild mottling of Columbian purple raspberries as red (raspberry) mosaic, at which time his concept (1) of that disease on black raspberries was of a rather severe type.

In 1931, a project on the maintenance of healthy black raspberry plantings was begun by the writer in southern Erie County in western New York. Columbian plantings are numerous in this district. Two types of mottling are present in them, that of the "mild mosaic" sort is universal; that of yellow mosaic is found only occasionally. It was deemed

essential to find out experimentally whether the mild type expressed the presence of a contagious virus, and, if so, to determine its identity and evaluate its menace to the healthy black raspberries.

Field Experiments and Observations

Etiology of Columbian "Mild Mosaic." During the growing seasons of 1932 and 1933, 5 series of transmission tests on black raspberries were carried out under the conditions and with the results given in table 1.

TABLE 1.—*The transmission of "mild mosaic" of Columbian hybrid raspberry plants to healthy Cumberland and Plum Farmer black raspberry plants, using Amphorophora rubi^a as the vector.*

Series and date of inoculation	Variety	Location and conditions	Treatment	No. of plants	Infections red (raspberry) mosaic	
					No.	Type
I June 28, 1932	Cumber- land	In commercial field; fair vigor; un- caged. ^b	Inoculated Check	4 5	4 1	4D ^c
II June 28, 1932	Cumber- land	In commercial field; good vigor; un- caged.	Inoculated Check	6 5	5 0	2C; 3D
III June 14, 1933	Cumber- land	In experimental plot; exceptional vigor; caged; pruned.	Inoculated Check	5 5	5 0	5D
IV June 14, 1933	Plum Farmer	In experimental plot; exceptional vigor; caged; pruned.	Inoculated Check	5 5	4 0	4E
V June 14, 1933	Cumber- land	In experimental plot; extra vigor; un- caged; unpruned.	Inoculated Check	6 6	5 0	1A; 3B; 1C

^a Aphids were taken at random from the three most vigorous Columbian plantings available and where all plants showed only mild mottling. Aphids for Series I came from one field; for Series II from a second; for Series III, IV, and V from a third.

^b Cages of cheesecloth were placed over plants in Series III and IV to prevent accidental infections.

^c As to degree of symptoms exhibited in a period two to sixteen months after inoculation:

A = mild mottling only.

B = medium mottling only.

C = medium mottling; slight necrotic effects on stem tips.

D = medium to severe mottling; decided necrosis on stem tips and leaf petioles.

E = severe or "blistered" mottling; severe necrosis and stunting.

Collections of *Amphorophora rubi*, the most effective aphid vector of raspberry mosaics, were made at random in 3 separate commercial plantings of Columbian raspberries. These source plantings were selected for excellent vigor and uniform mild foliage mottling. From the collections 6 to 20 aphids of varying ages were placed on each healthy test plant. Black-raspberry plants were chosen for inoculation because mosaic symptoms are most clearly and diagnostically expressed on them. Two standard black-raspberry varieties, Cumberland and Plum Farmer, were used. The former is lower in klendusity but higher in resistance to mosaic infection than is the latter.

In total, 23 of 26, or 88.5 per cent, of the black-raspberry plants exposed experimentally to infection by the characteristic "mild mosaic" of the Columbian purple raspberry became infected with red (raspberry) mosaic, according to the Rankin concept of that disease (10, 11). Only 1 of the 26 check plants became infected with red (raspberry) mosaic. This single accidental infection was not unexpected, as both inoculated and check plants in Series I, where it occurred, were exposed to and in no way protected from the natural spread of viruses.

To obtain confirmatory evidence, the order of the above experiments was reversed in 1934. Aphids (*Amphorophora rubi*) were reared under a cheesecloth cage on a Plum Farmer black-raspberry plant that was severely affected with typical red (raspberry) mosaic, the virus having come originally from an infected Ontario red-raspberry plant. Aphids of all ages were taken from this source and 10 were placed on each of 7 vigorous but mildly mottled Columbian plants growing in a commercial field. At the end of 2 months none of the Columbian plants thus exposed to infection exhibited symptoms different from the adjacent check plants. Presumably, then, the inoculated plants already contained the red (raspberry)-mosaic principle and further injection of it produced no apparent consequences.

These experimental results would seem to demonstrate conclusively that the "mild mosaic" mottling in Columbian purple raspberries is actually an expression of infection by a contagious mosaic virus and that this virus is the same as the red (raspberry)-mosaic virus common in black raspberries.

That such is the case has been verified by numerous field observations of natural spread. One decisive example of natural spread of red (raspberry) mosaic from Columbian raspberries into healthy black raspberries is given in the map in fig. 1. In this instance, in the spring of 1931, a 500-lot of virus-free Shuttleworth black-raspberry plants and a similar lot of "mild mosaic" Columbian purple-raspberry plants were set in adjacent plantings. No other sources of mosaic inoculum were present in the

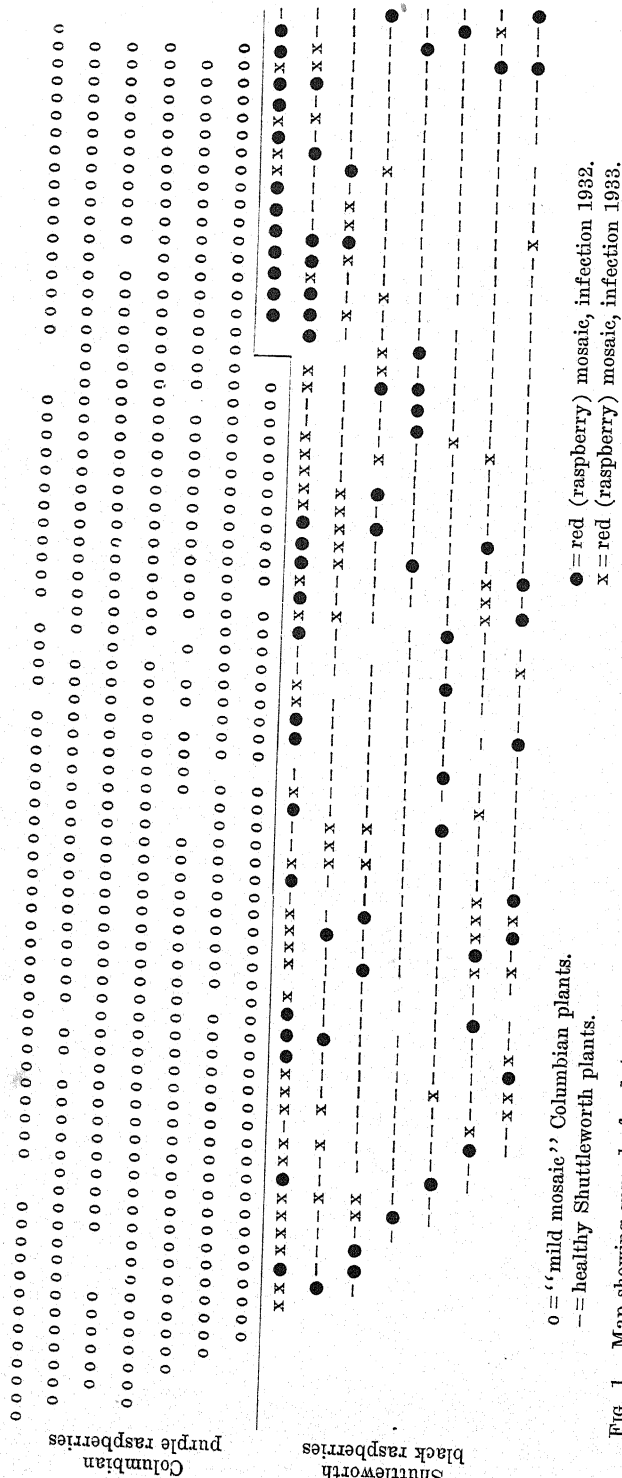


Fig. 1. Map showing spread of red (raspberry) mosaic into a planting of originally virus-free Shuttleworth black raspberries from an adjacent planting of Columbian purple raspberries uniformly affected with "mild mosaic" only. Both varieties planted in May, 1931; records taken June 15, 1932, and July 13, 1933. Farm of Borthwick and Hall, Silver Creek, New York.

vicinity. June 25, 1932, 73, or 15 per cent, of the 487 living Shuttleworth plants had contracted red (raspberry) mosaic according to the Rankin concept. These were rogued from the field by the growers. One year later, 81, or 20 per cent, of the remaining 414 black-raspberry plants were affected. Of 17 plants in the short row nearest the Columbians, 16 became infected during the two seasons of exposure. In the first long row adjacent to the Columbians, 54 of 71 plants contracted the disease. The distribution of this spread and the rate at which it occurred demonstrated that the "mild mosaic" Columbian plants were the source of the red (raspberry)-mosaic virus. Fields of the variety in general would seem to represent serious menaces to any near-by plantings of virus-free raspberry stock.

Varying Reaction of Black Raspberries to the Columbian "Mild Mosaic" Virus. A development of broader significance, however, was noted in this field experience. Here, to judge by the uniformity of mild symptoms displayed on the host Columbian plants, there could have been present but one common source of but one mosaic virus. Yet all degrees and phases of raspberry mosaic, other than yellow mosaic, were represented in the black-raspberry plants that became affected!

By referring to the last column in table 1 it will be seen that a similar phenomenon occurred in the black-raspberry plants experimentally inoculated with "mild mosaic" from Columbian plants. Of the 23 plants in these tests that developed infection, 1 extremely mild, 3 mild, 3 medium, 12 severe, and 4 extremely severe cases of red (raspberry) mosaic were observed.

The 4 Plum Farmer plants experimentally infected in Series IV were injured most severely of all. In field observations this variety has been noted as susceptible in the extreme to the red (raspberry)-mosaic virus.

The more resistant Cumberland plants used in the other 4 series varied in reaction. This variability seems traceable to growth features. In Series I subject plants were growing in a sandy soil. Their vigor had been average but, at about the time of inoculation, a prolonged summer drought and too deep cultivation with consequent root pruning brought their growth to a sudden stop. The type of infection obtained was uniformly severe for the variety. In Series II subject plants were in originally better vigor than those in Series I and, although affected by drought, had superior culture. Apparently, as a consequence, injury from infections here was somewhat less severe and less uniform than in Series I.

In Series III inoculated plants were on extra fertile soil and grew with extraordinary vigor and rapidity throughout the spring and early summer. Cheesecloth cages were placed over them in early June prior to inoculation and left until October. The shading of cages is conducive to abnormally

rapid growth and succulence in raspberry plants. Three weeks after inoculations in this series extremely mild mottling was evident on 2 leaves near the tip of only one lateral on one plant. At this time it was necessary to prune the plants in order to keep them within the confines of the cages. When the sublaterals produced from axillary buds subsequently had grown to a few inches in length, all of them on all the plants were necrotic on their tips and the mottling of their leaves was pronounced. The pruning, of course, had brought an abrupt check in the rank growth. It is thought that this allowed the virus time to reach and multiply in the growing points of the subject plants in uniform concentration and converted what would probably have been extremely mild (A degree) cases into severe (D degree) cases. The same pruning was done in Series IV and probably was responsible for additional and uniform severity of infection there on the more susceptible Plum Farmer plants.

In Series V the plants used were of the Cumberland variety, were growing in the same row, and, except for caging, were under conditions identical with those in Series III. Their growth, though not so rapid as in Series III, was unusually and steadily vigorous all summer. The pruning of laterals was not practiced on them. Here, one plant showed extremely mild mottling on one lateral in September and the next June it was a medium (C) case. Not until the spring following inoculation did the other 4 plants infected show symptoms and these were extremely mild (A) and mild (B) in degree of severity.

Since the inoculum was obtained in all cases where Columbian plants were affected uniformly with the mild mottling and where no other mosaic symptoms were present, it would seem improbable that more than one virus was concerned or that there could have been present several strains of one virus varying in virulence. The most probable conclusion has seemed to be that only one virus was involved and that the varying reactions obtained were due to varietal differences and to the influence of environmental conditions as they affected growth of each individual inoculated black raspberry plant.

CONCEPTS OF RASPBERRY MOSAICS

To understand the application of the above evidence on the identity of the "mild mosaic" in Columbian hybrid raspberries to the broader question of the identities of raspberry mosaics in general, it will be necessary to develop the entire issue by a chronological review of literature.

Review of Literature

From studies made in Ontario, Canada, on red raspberries, Rankin, Hockey, and McCurry (12), in 1921, presented symptom studies and field experiences that first separated mosaic as a virus-disease entity distinct

from other raspberry maladies. Wilcox and Smith (14), working in northern Ohio, in 1924 reported the first experimental inter-specific transmission of a mosaic virus, from red to black raspberries. Inoculation of this virus into black-raspberry plants produced wilting, stunting, and necrosis of tips with rather severe mottling. For the next several years, this reaction of black raspberries was adopted by investigators as the type of what came to be called "red-raspberry mosaic." In 1926, Dodge and Wilcox (7) described 1 type of mosaic on red raspberries and 3 types on black raspberries without naming them specifically. In March, 1927, Rankin (9), while commenting on varying symptom types in black raspberries, still discussed investigations with mosaic of raspberries in New York from the general standpoint of one disease.

Bennett (1), in Michigan, in May, 1927, used a classification of 3 mosaic types applied to all 3 species of raspberry, and called the types mild mosaic, red-raspberry mosaic, and yellow mosaic. Two years later, he (3) suggested that perhaps "a larger number of viruses than had heretofore been recognized" were responsible for the mosaic disease complex of raspberries. This suggestion was based on a great diversity in symptom expressions obtained in experimental transmission tests. Yellow mosaic was regarded as a distinct type, with mild and red-raspberry mosaics as intergrading extremes of another general type. Rankin (10), in 1930, published an account of field studies in New York in which a correlation of symptoms indicated that mild and red (raspberry) mosaics in black raspberries were caused by the same virus. In the same year, from observations in Michigan and Ohio, Bennett (4) described 4 types of raspberry mosaic, *viz.*, mild, medium, severe, and yellow, but stated that the number of viruses involved had not been determined. In a bulletin on virus diseases of black raspberries in 1931, Rankin (11) made a formal classification of raspberry viruses in which 3 mosaic viruses were listed, *viz.*, red (raspberry) mosaic, yellow mosaic, and mild mosaic. He expressed doubt, however, as to the existence of a distinct virosis that should be called mild mosaic.

In 1932, Bennett (5) published results of experimental transmission tests made in Michigan with 3 selected types of raspberry mosaics other than yellow. These were classified as mild red-raspberry mosaic, medium red-raspberry mosaic, and severe red-raspberry mosaic. Each type was reported to have remained stable in degree of symptom expression through series of transmissions on red (variety King) and black (variety Cumberland) raspberries during a period of 3 years. The possibility of several strains of one virus with varying virulence was suggested to account for the results obtained.

In a general treatise on raspberry diseases, Rankin (5), in 1933, without presenting further evidence or discussion, followed a two-virus classification, namely red (raspberry) mosaic and yellow mosaic.

Some salient points are worth comment. All investigators are agreed on the individuality of the yellow-mosaic virus. The controversy, then, resolves itself into whether one or more than one virus is involved in the production of the wide range of mosaic symptoms on raspberries known not to be due to the yellow-mosaic virus. Argument is possible over this question because the said range of symptom extremes extends from a very light mottling visible only early in the season and accompanied by no appreciable injury *through all gradations* to a "blistered" mottling associated with severe necrotic, growth-stunting, and rapid-killing effects. Complicating factors, such as weather, plant vigor, three species of hosts with numerous varieties, and possible association with the yellow-mosaic virus, all affect symptom expression and thus increase the chances for confusion and contradictory opinions as to causal relationships. One investigator may explain satisfactorily that a single virus causes all the varied symptoms and that their diversity is due to varietal and individual plant susceptibility or reaction, as modified by weather, soil, and growth factors. Another may contend with equal plausibility that, since the diversity of reaction is often great within even a single planting of one variety, the different types of disease could be produced only by different viruses or combinations of viruses, or at least by distinct strains of the same virus differing in virulence.

One highly significant point is that, although the range of severity of symptoms is wide, it is *graduated*. Bennett (3, 5) has twice mentioned the lack of clear demarcations between his selected types of mild, medium, and severe red-raspberry mosaics. Apparently 4, 5, or a dozen representative types of disease severity may be selected as well as 3. The number of divisions to be made would seem to depend on the investigator's desire for detail rather than on the existence of so many distinct types in nature.

Another interesting feature is the evolution that the application of the term red-raspberry mosaic, or its other forms, red (raspberry) mosaic and red mosaic, has undergone from an originally restricted scope to its present broad usage. Regardless of their opinions as to the number of viruses involved, all investigators are now using this name to include all types of mosaic in raspberries other than yellow mosaic.

Application of the Columbian "Mild Mosaic" Studies

The present experiences with "mild mosaic" in Columbian hybrid raspberries appear to have demonstrated not only that this mosaic is the red (raspberry) mosaic, but that one strain of this one virus may produce the entire range of other-than-yellow mosaic symptoms that have been observed on black raspberries in eastern North America. These findings support the Rankin theory (10, 13) that there are but two known rasp-

berry mosaic viruses in this region, namely, red (raspberry) mosaic and yellow mosaic. When put to test in extensive field work, this concept has proved satisfactory and fitting in every situation.

Final and indisputable decision as to the number of raspberry-mosaic viruses cannot be made, of course, until the viruses can be isolated and studied *in vitro*. But there is no immediate prospect of this being possible as they seem to perish almost simultaneously upon extraction. As long as this situation exists, the opinions of investigators, even though drawn from experimental inoculation trials, will be based necessarily upon their observations of symptoms and interpretations thereof. And such opinions, of course, will be open always to question.

However, practical dealings with raspberry mosaics require the formation of definite concepts. For the present, then, the general adoption of the 2-virus classification is suggested, as it not only appears to be the more accurate but possesses the additional advantages of being simpler and clearer.

PROPOSED CHANGE IN TERMINOLOGY

Although contradictory explanations still may remain as to why the differences in degree of symptom severity exist in red (raspberry)-mosaic infections, as pointed out in the literature review above. American investigators are now using essentially the same name for the disease. But the term "red-raspberry mosaic" and all its derived forms seem objectionable in several respects, especially to those persons not intensively acquainted with raspberry virus diseases.

"Red-raspberry mosaic" is not only a cumbersome name, but it is misleading. This particular mosaic is not limited to nor is it of most common occurrence in red raspberries; in fact, it is more frequent and more distinct in black and purple than in red raspberries. The term "red-raspberry mosaic" was derived originally from the interspecific transmission of the virus from red to black raspberries reported by Wilcox and Smith (14) in 1924. "Red mosaic" was introduced into literature by Rankin (10) in 1930 simply as an abbreviation of its predecessor. This name carries the same likelihood of misconstruction, *i.e.*, that the disease occurs principally in the red-raspberry species. In addition, it is mistaken frequently as having reference to a diagnostic color feature in the disease symptoms. Such misunderstanding is apt to occur particularly because "red mosaic" seems contradistinctive to the name "yellow mosaic" that is descriptive of the disease in raspberries to which it is applied.

The discarded terms "mild mosaic," "medium mosaic," and "severe mosaic" (4) have been used as descriptive of degrees of what now appears to be one disease. No one of them alone satisfactorily denotes the entire complex of the disease.

There seems to be no reason to continue the use of any of these names in view of their inappropriate connotations. *Green mottle mosaic* is suggested here as the most logical and suitable substitution for them. This name is descriptive of the fundamental symptom of the disease, all species and varieties of raspberries considered. With this disease, the severity of mottling is known to vary extremely but, whenever shown, consists of a contrast between light green and dark green areas in the leaves. The only known exception to this general rule is to be found occasionally on fruit-spur foliage of seriously injured individual plants of a very susceptible black-raspberry variety like Plum Farmer. In such restricted instances the foliage may be a solid green but of much deeper intensity than normal.

The name "green mottle mosaic" also portrays the general contrast in chlorotic symptoms that distinguishes the 2 raspberry mosaic diseases. A true mottle pattern is characteristic of green mottle mosaic, while the chlorosis, evident in yellow mosaic, is not strictly a mottling but a general yellowing of tissues that affects small portions of leaves or entire leaves. This yellowing is progressive in individual leaves from their veins outward and their margins inward.

The yellow mosaic virus tends to produce more leaf deformity in all species of raspberries, but this difference is not constant. There is another difference between the 2 diseases in the position of leaves on affected plants in which chlorosis appears. Green mottle mosaic is seen most commonly in a host plant on the lower leaves of the spurs on fruiting canes and on the lower leaves of the turions, whereas yellow mosaic is expressed on the upper leaves of the fruit spurs and on the higher leaves of turions. This position difference, however, is relative and intergrading and varies between seasons and varieties according to weather, host growth conditions, and length of time plants have been infected. Neither host position nor leaf-deformity differences are deemed suitable criteria for founding distinctive names for the raspberry mosaic diseases. Besides, renaming on either of these bases would require redesignation of both diseases.

CONCLUSION

In brief, it is believed that the situation in regard to raspberry mosaics would be simplified and clarified for many and would be presented more accurately according to the results of experimentation and observation up to the present time in eastern North America if the following classification of them were adopted.

Raspberry Mosaics:

Virus 1. Green Mottle Mosaic. This name is recommended as a substitute for the terms "red-raspberry mosaic" and "red mosaic." It is

meant to include the entire range of symptom expression from very mild to very severe cases; in fact, all types of mosaic of raspberry other than the distinct cases known to be due to the yellow-mosaic virus. For convenience, the term "green mottle mosaic" might well be contracted to "green mosaic."

Virus 2. Yellow Mosaic. All investigators are agreed upon the usage of this name. No change is needed or advised.

SUMMARY

Experimental transmission trials and field studies in disease spread have proved that the "mild mosaic," universally present in Columbian hybrid raspberries, is caused by a virus identical with the red (raspberry)-mosaic virus.

In these tests, the inoculated black-raspberry plants developed all degrees of other-than-yellow mosaic symptoms from extremely mild to extremely severe phases. Since the mosaic inoculum was obtained from sources where its presence had been uniform in expression, the results were interpreted as demonstrating that a single virus may produce all the mosaic types other than yellow mosaic that occur in black raspberries in eastern North America. This indication has been substantiated by field experiences.

Knowledge of the environmental, climatic, and cultural conditions that affected the black raspberry plants inoculated in these experiments indicated that such factors, through their effect on host growth, influenced the severity of reaction obtained in individual cases.

These findings support the Rankin theory that there are only two viruses involved in raspberry mosaics of eastern North America, namely, red (raspberry)-mosaic and yellow-mosaic.

The name "red-raspberry mosaic" and its derivatives are confusing in connotation. The much more fitting term *green mottle mosaic* is suggested as a substitute. For the sake of brevity, this name might be shortened to "green mosaic."

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THE PARASITISM OF ARCEUTHOBIMUM (RAZOUROWSKYA) CAMPYLOPODUM ON PINUS JEFFREYI

JEAN DUFRÉNOY

(Accepted for publication February 27, 1935)

Histological studies of the relations between the parasitic Lorantheae and pines date back to 1905, when Peirce¹ studied the germination of *Arceuthobium (Razourowskya) occidentale* on *Pinus radiata*. He showed that when the root tip of the germinating embryo comes in contact with a slight elevation of the surface or the base of a bundle of needles, it forms a holdfast consisting of a mass of parenchymatous cells, which penetrates, haustorium-like, and branches into the medullary rays of the host.

Vascular tissues are formed at an early stage, and Peirce has shown the "direct contact of a tracheid of the parasite with a tracheid of the host;—in this perfect connexion of the xylem-tissues of parasite and host we have nothing uncommon."

The writer found the same relationship (Fig. 1) between tracheids of *Arceuthobium (Razourowskya) campylopodum* and the tracheids of *Pinus jeffreyi*, in material made available through the courtesy of Dr. E. P. Meinecke. The haustorium of the parasite, pushing into the medullary ray of the host, causes hyperplasia of the cells of the medullary ray, and inhibits the normal differentiation of the xylem elements in the immediate vicinity. Instead of perfectly parallel elongated tracheids showing the normal bordered pits, elements are formed with irregular woody thickenings of their walls.

Figure 1 shows, in a radial section of an infected twig of *Pinus jeffreyi*, the xylem of *Arceuthobium* (R) descending from the upper parts of the parasite through a medullary ray (M) of the pine, there to end in close contact with the xylem elements of the pine wood.

Peirce emphasized the relations of the phloem tissues of *Arceuthobium* with those of pine, but he "sought vainly for anything more than contact between the cells of *Arceuthobium* and the sieve tubes of pine." Neither did the writer find anything more in Jeffrey pine, nor did he expect it. *Arceuthobium*, like other such parasites, shows little histological differentiation; besides, it is felt that in the parasitic relationship, as well as in the whole problem of translocation of materials through the vascular plants, the anatomical and histological aspects have been overemphasized to the point of overshadowing the physiological side. What we are concerned

¹ Peirce, George J. The dissemination and germination of *Arceuthobium occidentale* Engl. Ann. Bot. 19: 99-113. Jan. 1905.



FIG. 1. Section through a twig of *Pinus jeffreyi*, showing the xylem of *Arceuthobium campylopodum*, R, in close contact with the pine wood-elements, X. All lignified tissues, staining red with safranin, are shown in solid black. Bordered pits are outlined. M, nonlignified portions of wood.

with is primarily a problem of relation between the two living organisms and, therefore, a problem of relationship between the living cells of both.

The problem should be studied in sections of living material. Free-hand sections, stained with a solution of neutral red, prepared in the Ringer fluid, plainly show the importance of living parenchymatous cells where the parasite comes into contact with the host. The main effect of the parasite is to induce the formation of wide medullary rays, thereby reducing the formation of wood vessels.

The parasite itself is made up of a mass of parenchymatous cells, with merely an axial row of elongated cells, showing lignified thickenings

of the cell walls and representing rudimentary wood vessels. Elongated living cells, surrounding these wood vessels, may be interpreted to represent the phloem tissues. Each living parenchymatous cell, either of the parasite or of the host, shows one or a few vacuoles, containing a watery solution that stains red with neutral red. Where the haustorium comes into contact with the tissues of the pine, each cell shows a number of small vacuoles. (Figs. 2 and 3.) The presence of many small vacuoles in a cell is correlated with an active translocation of soluble materials, and generally with that of polypeptides.

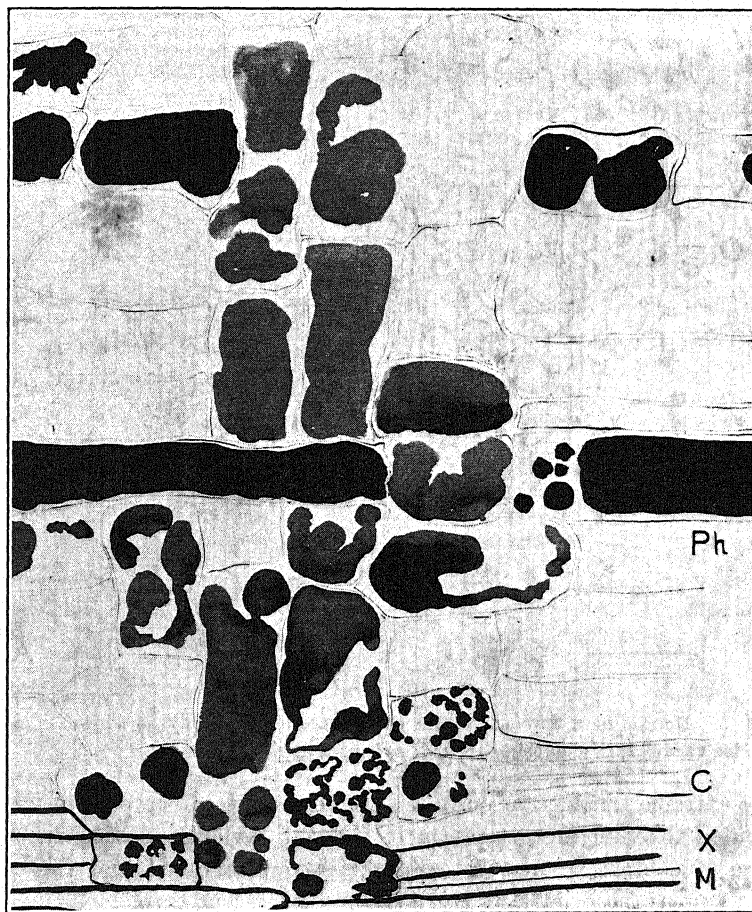


FIG. 2. Section of twig of *Pinus jeffreyi*, showing phloem Ph, cambium C, xylem X, and medullary ray M, penetrated by the cells of *Arceuthobium*. Small vacuoles, the contents of which are stained red by neutral red in the living cells, are shown in black; vacuoles staining pink are shown in grey.

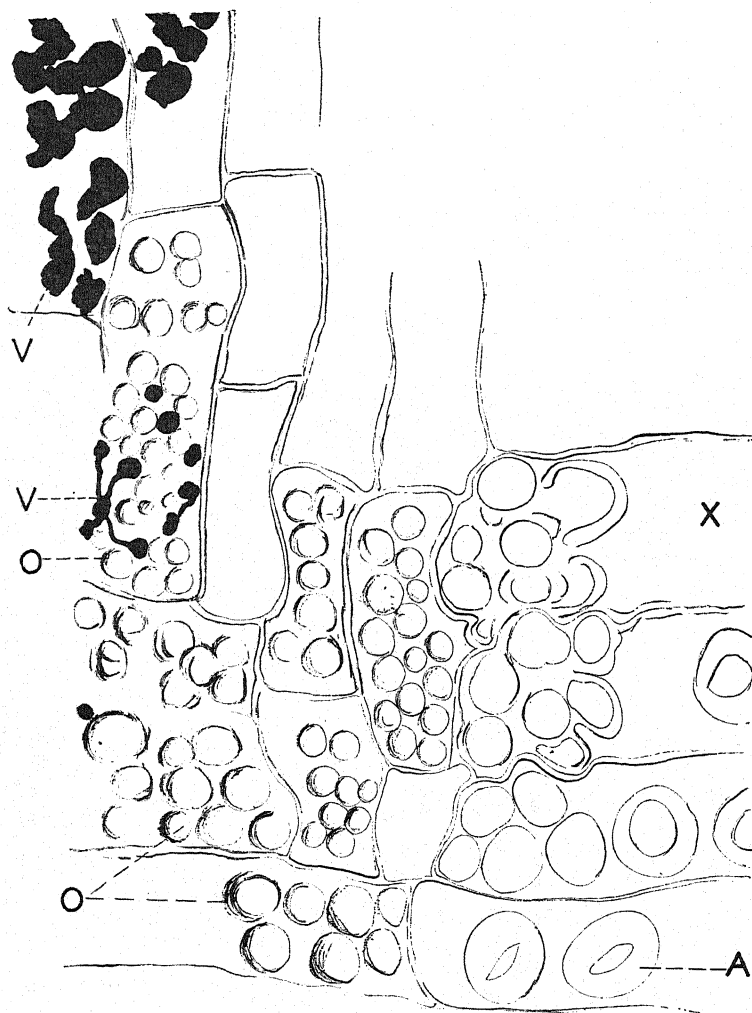


FIG. 3. Details of a few cells from the same medullary ray as shown in figure 2. V indicates vacuoles, O oleoresin drops, X xylem, A bordered pits.

The extreme fragmentation of the vacuolar material into many droplets distributed through the cytoplasm in the cell obviously results in a great increase of contact surface between the cytoplasm itself and the surface of the vacuolar material. From the results of recent cytological investigations it appears that most of the activities of the living cell, particularly the respiration process, are mainly located at those surfaces of contact between the cytoplasm and the vacuoles, so that the greater the surface of contact, the more active the cell.

We may, therefore, infer from the cytological aspects of the living cells that, where a haustorium of *Arceuthobium* comes in contact with the medullary rays of the pine, an active translocation of nutrients takes place, presumably as the result of enzymatic activity by the cells of the haustorium.

The cells of the infected medullary rays are rich in droplets of oleoresins. The tissues of the *Arceuthobium*, imbedded in the cortex of the pine branch, as examined in the month of February, show an abundance of starch. This may, in part, explain why porcupines are especially fond of feeding on them.

PONT-DE-LA-MAYE (GIRONDE), FRANCE.

(STATION DE PATHOLOGIE VÉGÉTALE).

A PROTECTIVE ZONE IN RED GUM FIRE SCARS

GEORGE H. HEPTING AND DOROTHY J. BLAISDELL

(Accepted for publication March 25, 1935)

INTRODUCTION

Of several southern hardwood species studied in a recent pathological investigation in the Mississippi Delta, red gum, *Liquidambar styraciflua*, and persimmon, *Diospyros virginiana*, appeared to be the most resistant to decay infection through fire scars (Table 1). Shortly after being scarred

TABLE 1.—Percentage of fire-scarred trees decayed in several southern hardwoods^a

Species	Number of trees examined	Percentage of trees decayed
Hackberry (<i>Celtis laevigata</i>)	23	100
Nuttall Oak (<i>Quercus nuttallii</i>)	33	82
Overcup Oak (<i>Q. lyrata</i>)	10	80
Water Oak (<i>Q. nigra</i>)	32	72
Ash (<i>Fraxinus</i> spp.)	19	47
Red Gum (<i>Liquidambar styraciflua</i>)	85	42
Persimmon (<i>Diospyros virginiana</i>)	5	20

^a A tree was considered decayed if rot had progressed $\frac{1}{2}$ in. or more in from scar surface. As a rule a tree designated as decayed was definitely decayed, as in figure 1, B. All trees were scarred 8 years before dissection and at no other time before or after that date.

by fire both of these species form hard dark zones on the surfaces of the scars, extending to a depth of about 2–10 mm. into the wood. Other species, particularly ash, *Fraxinus* spp., also tend to form such zones but the zones are not so well developed as in red gum and persimmon. These zones appear to offer a protection from decay to the wood beneath. The protective zone in red gum was studied in detail.

MACRO- AND MICROSCOPIC APPEARANCE OF PROTECTIVE ZONE

Figure 1, A, shows a section through a young red gum scarred by fire 22 years and again 15 years before dissection. The dark protective zone is plainly visible around the edges of the scars. Even though the scars on this tree exposed a considerable portion of the sapwood at the base, the tree remained sound. In a manuscript¹ by the senior author shortly to be published, it is shown that red gum is more subject to decay from fire scars if

¹ Unpublished manuscript "Decay Following Fire in Young Mississippi Delta Hardwoods" by George H. Hepting.

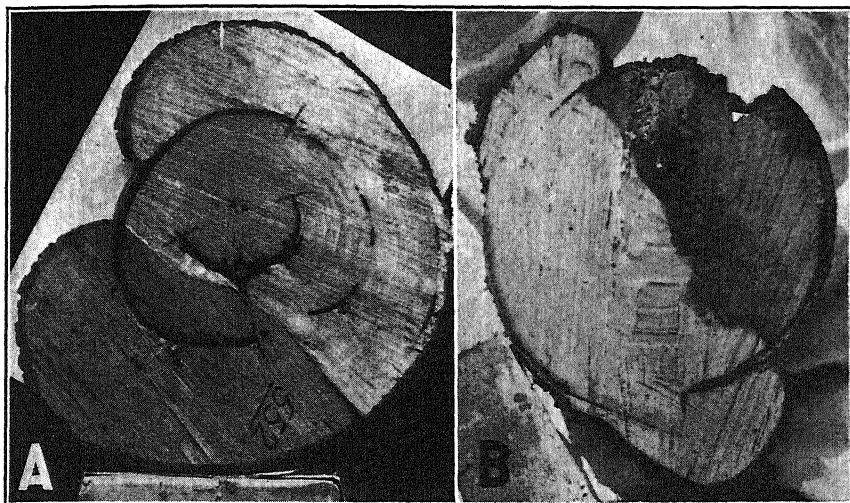


FIG. 1. A. Cross section through base of a young red gum scarred by fire 22 years, and again 15 years before dissection. $\times \frac{1}{8}$. Note protective zones and absence of decay behind scar. B. Cross section through base of young red gum scarred on one side 4 years and on the other side 2 years before dissection. $\times \frac{1}{8}$. Note disruption of protective zone on one side, and decay of sapwood behind scar.

burned more than once than if burned only once. Figure 1, B, shows a section through a young red gum scarred on one side 4 years and on the other side 2 years before dissection. In spite of the relatively short time since this tree had been scarred, there was considerable decay of the sapwood behind the older scar. Note that while the protective zone is plainly visible it has been broken at one place.

The protective zone in red gum fire scars usually comprises several of the outer layers of sapwood cells, although it may be formed some distance in from the surface. The zone appears similar to normal heartwood except that it is darker, much harder, and is heavily infiltrated with a brown substance that appears to be a gum and will be referred to as gum in this article.

Sections were prepared of the protective zone, and these were examined under the microscope. There was about one millimeter of sapwood on the face of the block in which no gum was deposited. The gum zone was behind these cells. This was about 2 millimeters in extent in the sections examined. Behind this zone was sapwood in which no gum was found in the wood fibers and tracheids. Gum had, however, been produced in the medullary-ray cells and in some of the wood-parenchyma cells for a considerable distance from the zone proper. The cells within the zone were heavily impregnated with a dark brown gum (Fig. 2, A, and C). The ray and wood-

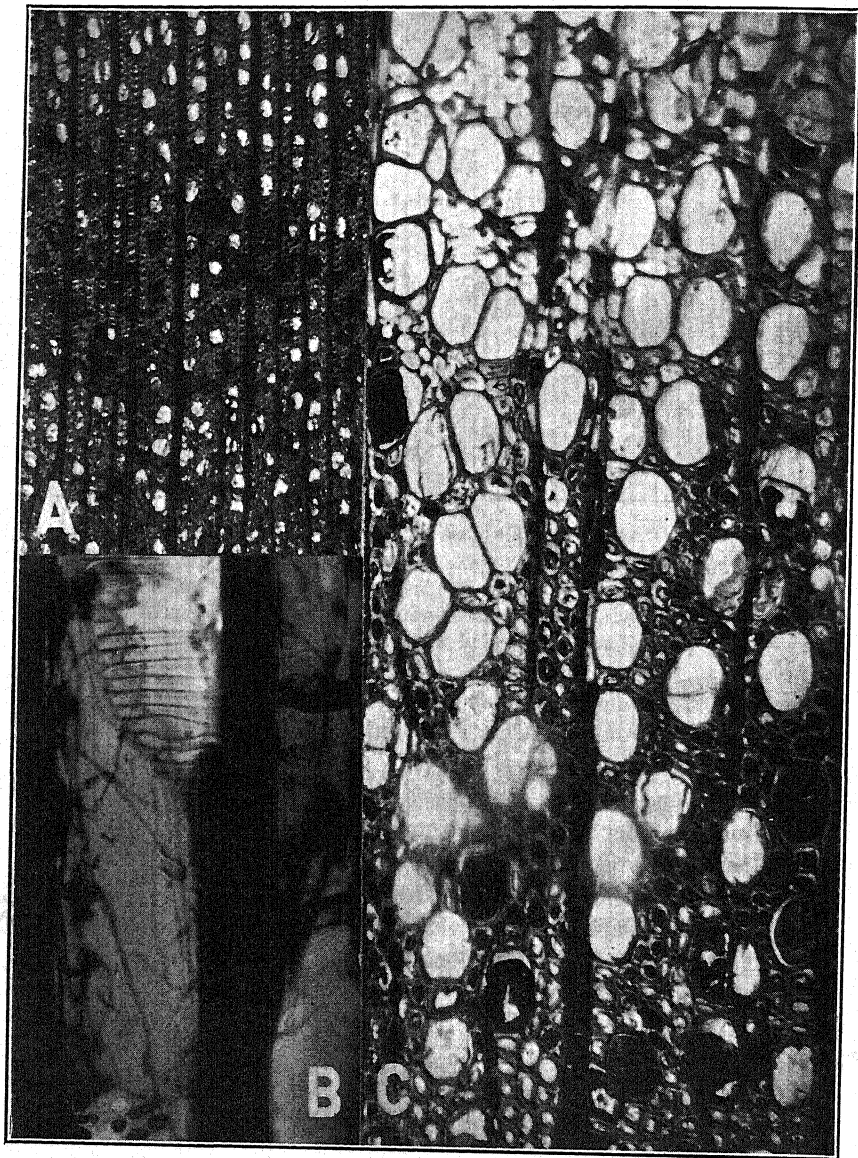


FIG. 2. A. Cross section taken at outer edge of red gum fire scar, showing protective gum zone formed just back of scar edge and normal wood beyond gum zone. The scar edge is at bottom of A. B. Mycelium of *Polyporus pargamenus* in vessel and parenchyma cell within gum zone. C. Cross section taken at border of gum zone (lower part) and originally normal sapwood (upper part) after block had been subjected to decay by *P. pargamenus* for one year. Note decomposition of cell walls in originally normal wood and their preservation within gum zone.

parenchyma cells were completely filled with gum in the densest part of the zone and the lumina of the fibers were nearly filled. Even the largest vessels usually contained considerable gum, this being found as a hard brown covering or thick film over the inner cell walls in some and in others in the form of globules. (Fig. 2, B, lower end of vessel.)

RESISTANCE OF PROTECTIVE ZONE TO DECAY IN ARTIFICIALLY
INOCULATED BLOCKS

Several small blocks about $\frac{1}{4}$ in. square and $\frac{1}{2}$ in. long were cut from the outer part of a red gum fire scar. The blocks included part of the protective zone and about $\frac{1}{4}$ in. of sapwood adjacent to and behind it. These blocks were placed in test tubes with water and autoclaved for $\frac{1}{2}$ hr. at 15 lbs. pressure. *Polyporus pargamensis* Fr., growing on agar, was placed on some and on others *P. gilvus* (Schw.) Fr. was planted. These 2 fungi were used, since they are well-known rotters of dead sapwood. The tubes were then allowed to stand for a year at room temperature. At the end of that time the blocks were examined. A dense fungous mat completely covered each of the blocks. Upon removal of the mat it was found that the previously normal sapwood was completely decayed both by *P. pargamensis* and *P. gilvus* so that it crumbled under slight pressure between the fingers. The adjacent gum-filled zone, however, appeared to be as hard and firm as it had been prior to inoculation.

The decayed blocks were placed in an alcohol-formalin-acetic fixing solution. After remaining in this solution for 48 hours or longer the blocks were dehydrated by passing them through a series of butyl alcohol and infiltrated with paraffin.² The blocks were then embedded in paraffin and the paraffin blocks were soaked in water to facilitate cutting. Sections were cut on a rotary microtome. Some of the sections were stained with Fleming's triple stain and others with the methyl violet-Bismark brown stain.³ The latter stain was found to be the most effective. Sections of blocks inoculated with *Polyporus pargamensis* or *P. gilvus* were compared with each other and with those from noninoculated blocks. Both fungi completely decayed the sapwood adjacent to the protective zone.

Upon examination of the sections from blocks that had been subjected for a year to decay by each of the two fungi it was found that neither the position nor extent of the protective zone had changed. There seemed to be a little less gum in the cells in some of the sections of decayed wood but, since this was not so in all of them, the loss of gum may have been due to the

² Zirkle, Conway. The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science n.s., 71: 103-104. 1930.

³ Hubert, E. E. A staining method for hyphae of wood-inhabiting fungi. Phytopath. 12: 440-441. 1922.

embedding or staining processes. The cells in the gum region showed no signs of decay and appeared to be in as good condition as in undecayed wood. The cells beyond the gum area, however, were very much decayed (Fig. 2, C). In most cases the tertiary and secondary thickenings of the cell walls had almost, if not entirely, disappeared, only the middle lamellae of the cells being left. In some places the middle lamellae had become so thin and brittle that the connections between cells had become entirely obliterated, only fragments remaining. The ray cells and the wood-parenchyma cells, which had been filled with gum, were undecayed.

Considering the excellent conditions for decay and the relatively long time these small blocks were submitted to fungous action the gum-filled zone showed great resistance to the action of the two fungi used, in contrast to the readiness with which the normal wood decayed.

The fact that the cells in the gum-filled zone did not decay in the laboratory tests is not entirely explainable by any failure of the mycelium to penetrate this zone, because, as shown in figure 2, B, mycelium was abundantly present in many of the wood elements in this gum zone that were not completely filled with gum. The zone appears to be similar to heartwood that has been heavily infiltrated with gum. The resistance of this zone to decay by the fungi may thus be similar to the resistance of normal heartwood to the action of sapwood-rotting fungi, with the added mechanical resistance of the heavy infiltration of hardened gum. In the present tests the gum appeared not to be utilized by the fungi, as it was still abundant at the end of the incubation period of a year. In no case were hyphae found penetrating a gum globule or passing through a completely gum-filled cell. It is probable that, under natural conditions, fungus mycelium could penetrate all the way through the gum-filled zone only with great difficulty, if at all. Under the laboratory tests the fungi were permitted to become well established around the gum area and could readily enter the vessels in this area through their open ends. Under natural conditions the proximity of the zone to the outside surface of the scarred area would leave a fungus little normal wood in which to become established. Penetration to the underlying sapwood also would have to be in a radial direction through dozens of gum-filled cells.

The protective zone formed in a red gum fire scar, through its impervious nature resulting from heavy gum infiltration, apparently acts to preserve the living sapwood beneath the surface of the scars from drying and subsequent decay. Through its resistance to decay this zone may remain an effective seal for many years. Many cases have been observed where large red gum fire scars (Fig. 1, A) have completely healed without any decay development behind the scarred area. If a once-scarred red gum is exposed to more fires the sapwood beneath the protective zone may be killed

by heat, and decay may then readily take place (Fig. 1, B). The reason that the sapwood behind the protective zone decays more readily when killed may be because subsequent checking allows ingress of fungi behind the zone. It also may be that wood-destroying fungi are in contact with the wood beneath the zone while the zone is still intact, but are capable of decaying it only if the wood be dead. In some cases the sapwood beneath the protective zone may become exposed to decay through imperfect formation of the zone or through the action of insects, particularly ants and termites, which commonly infest scarred trees in the Delta Region. This zone also may act as a mechanical barrier preventing mycelium from reaching the sapwood beneath.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

PRESENT GENERIC STATUS OF THE CITRUS-SCAB ORGANISM

ANNA E. JENKINS

(Accepted for publication February 2, 1935)

Sphaceloma fawcettii Jenkins, the fungus that causes scab of citrus (*Citrus*), is undoubtedly to be classified in the genus *Sphaceloma* where it was placed (6) in January, 1925. It is the writer's understanding that this is now also the opinion of Doidge and Butler, although in September, 1924, they referred (4) the fungus to the genus *Sporotrichum* as closely related to the animal pathogens of this "heterogenous" group.

The genus *Sporotrichum* as amended by Saccardo (9) included two species, *S. virescens* Link and *S. roseum* Link. The former species is excluded by Lindau (8). *S. roseum* may, therefore, be regarded as the type, as is designated by Clements and Shear (3, p. 389), and *Sphaceloma fawcettii* is clearly not related to this species.

A recent parallel cultural comparison of *Sphaceloma fawcettii* with a culture of *Sporotrichum schenckii* (Hektoen and Perkins (5)) De Beurmann and Gougerot (1), the first animal pathogen to have been classified in the genus *Sporotrichum* (1, 10) suffices to demonstrate that the two are strikingly unlike, as shown below. The culture of the *Sphaceloma* employed was isolated from scab lesions on sour orange (*Citrus aurantium* L.) from Florida, on October 20, 1928, that of the *Sporotrichum schenckii* was contributed by Charles Thom, of the Bureau of Plant Industry, who obtained it from Rhoda W. Benham, of the Laboratory for Medical Mycology, College of Physicians and Surgeons, New York, N. Y. The cultures were grown on both Sabouraud's maltose agar and on potato-dextrose agar.

As illustrated in Figure 1, A and C, and D to F, they were distinctly unlike on both media, scarcely showing even a superficial resemblance. It will be noted that the 24-day-old culture of *Sphaceloma fawcettii* on the maltose agar (Fig. 1, A) does not cover the surface of the slant as does the *Sporotrichum* (Fig. 1, C), of which the growth was limited by the area of the slant. This culture and the similar one of *Sphaceloma ampelinum* de Bary, i.e., *Elsinoe ampelina* (de Bary) Shear, as the fungus is now known in its perfect stage, included in the comparison (Fig. 1, B) on maltose agar were both much brighter colored than the *Sporotrichum*; that of *Sphaceloma fawcettii* was Hay's maroon,¹ with the lighter portion on one side Prussian red, and that of the *Elsinoe*, mostly Vandyke brown, or of

¹ Ridgway, R. Color standards and color nomenclature. 43 pp., 53 col. pls. Washington, D. C., 1912.

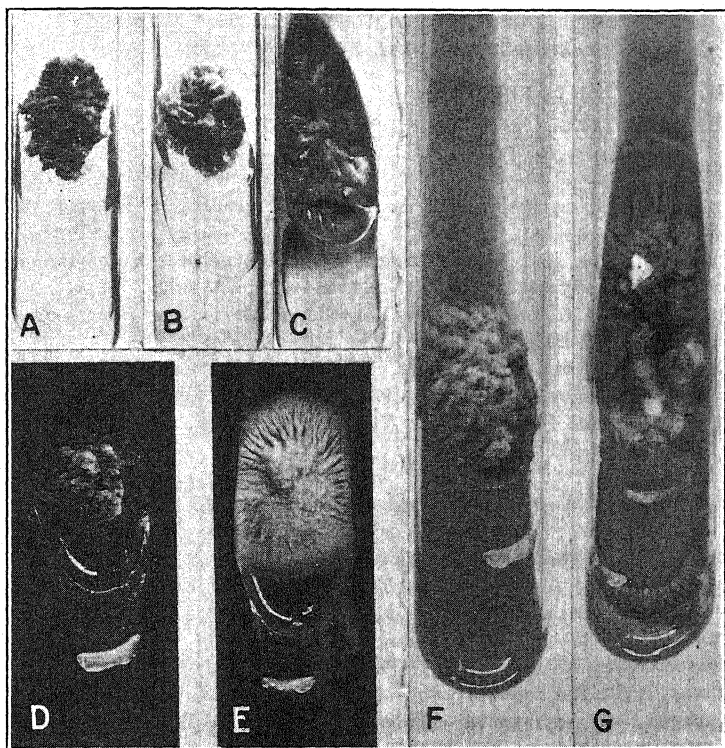


FIG. 1. Parallel cultures of different fungi. A-C. On Sabouraud's maltose agar. A. *Sphaceloma fawcettii*. B. *Elsinoe ampelina*. C. *Sporotrichum schenckii*. D and E. On potato-dextrose agar. D. *Sphaceloma*. E. *Sporotrichum*. F and G. Cultures shown in D and E, photographed at a later date. All $\times 1$.

this color mingled with Rood's brown, while the culture of *Sporotrichum* ranged from drab gray to black.

On potato-dextrose agar the young thallus of *Sphaceloma fawcettii* was raised and irregularly convoluted from the first, and it had scarcely reached the sides of the tube, even after 12 days' growth (Fig. 1, D). On the other hand, the somewhat faster-growing *Sporotrichum* began its development as an appressed smooth growth with an even margin and with no sign of convolutions for at least 7 days. Numerous radial convolutions developed, however, during the next 5 days (Fig. 1, E). The appearance of these 2 cultures had changed considerably by the time they were 28 days' old (Fig. 1, F and G), or even 2 months' old, but they were still unlike.

In a related paper (7) it is shown that *Elsinoe ampelina* is not pathogenic on rabbits, as previously reported by Charrin and LePlay (2, p. 521-523), and on the basis of the present contribution there is no reason to

believe that the citrus plant pathogen is closely related to such fungi as the human pathogen *Sporotrichum schenckii*.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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PHYTOPATHOLOGICAL NOTE

A Sphaceloma on Fruit of Hesperethusa crenulata, a Remote Citrus Relative from India.—Winston, Bowman, and Bach¹ reported *Clausena lansium* Skeels, of the tribe *Clauseniae*, as moderately affected by Florida citrus scab caused by *Sphaceloma fawcettii* Jenkins, and stated that, with this exception, the rutaceous plants affected by the disease belong to the tribe *Citrinae*. Among these they assert that "members of only 4 genera, viz., Citropsis, Poncirus, Fortunella, and Citrus, are known to be affected by this disease. Only one occurrence on Citropsis and Fortunella was noted and Poncirus was only moderately attacked. These authors explain that Fortunella and Poncirus were only recently removed from the genus Citrus."²

Hesperethusa crenulata (Roxb.) Roemer, a remote citrus relative of the tribe *Triphasinae* was not affected by scab in Florida where the work by Winston, Bowman, and Bach, referred to above, was done. But there is a previous record of scab on this host as present on fruits presented to Walter T. Swingle by the Curator of the Royal Botanical Gardens at Sibpur, near Calcutta, British India.³ These ripe fruits were received in February, 1916, under the Foreign Seed and Plant Introduction number 41947, and were labelled *Limonia acidissima* L. as *H. crenulata* had "commonly but erroneously" been called at that time.

This plant is said to be "native to dry hills in Ceylon, India, Burma, and Indo China."⁵ The more or less circular, raised or convex, and sharply delimited scab lesions are shown in figure 1. The causal fungus (*Sphaceloma* sp.) is visible as dark punctiform areas at the center of the lesions on the enlarged fruits (Fig. 1, B). The fructifications visible in figure 1, C

¹ Winston, J. R., J. J. Bowman, and W. J. Bach. Relative susceptibility of some rutaceous plants to attack by the citrus-scab fungus. Jour. Agr. Res. 30: 1087-1093. 1935.

² The botanical classification and nomenclature of the rutaceous plants referred to in this article follow Walter T. Swingle.^{a, b, c}

^a Swingle, W. T. Study of the phytogenetic relationships of the rutaceous sub-family Citrateae, including the citrus fruits and their wild relatives, with experimental studies in the hybridizing and grafting of plants of this sub-family. [In Japanese] Studia Citrologia 1: 1-4 (N. 1, March 1927).

^b Swingle, W. T. Citrus and related genera. In Bailey, L. H. The Standard Cyclopedia of Horticulture. New York. See V. 1, p. 91 (1914), V. 3, p. 1478, fig. 1825 (1915).

^c Swingle, W. T. The name of the wood apple, *Feronia Limonia*. Jour. Wash. Acad. Sci. 4: 325-328. 1914.

³ U. S. Department of Agriculture Federal Horticultural Board Letter of Information No. 22, p. 6. 1916.

⁴ Loc. cit. See footnote c.

⁵ Loc. cit. See footnote b.

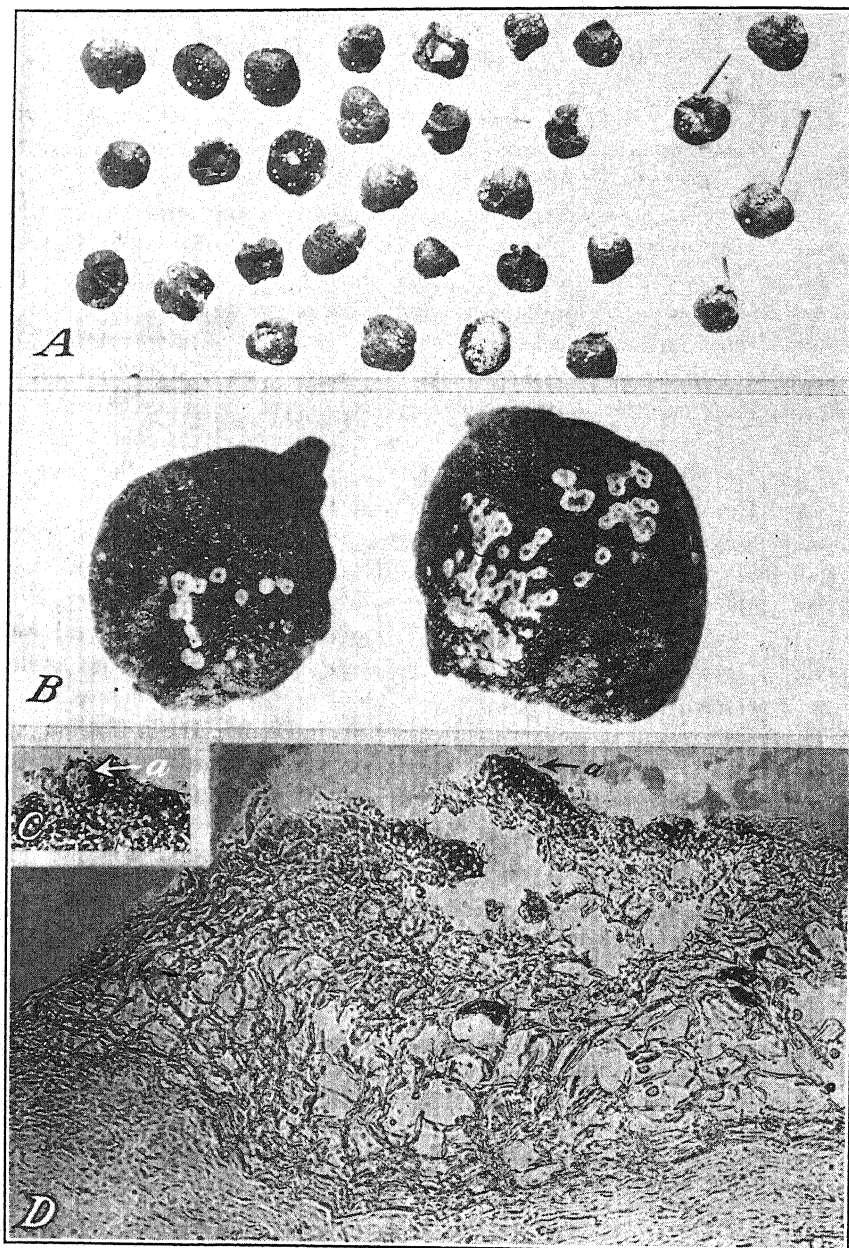


FIG. 1. *A* and *B*. Scabbed fruits of *Hesperethusa crenulata* from British India (*A*, $\times 1$; *B*, $\times 5\frac{1}{2}$). *C* and *D*. Sections showing (*D*) the hyperplastic character of the lesions and (*C*, *a* and *D*, *a*) fructifications of the *Sphaceloma* as explained in the text (*C*, $\times 310$; *D*, $\times 275$).

and *D*, consist principally of a mass of microconidia (Fig. 1, *C*, *a*) and acervuli (Fig. *D*, *a*).

Further critical study of this *Sphaceloma* is, of course, essential for its specific or varietal identification. The symptomatological characters have a certain similarity to those of sweet orange fruit scab, known only in South America and attributed to *S. fawcettii viscosa* Jenkins. This similarity is suggested by a comparison of figure 1, *B*, here shown with a closely corresponding illustration of sweet orange fruit scab previously published.⁶

For some time it has been thought advisable to search for *Sphaceloma* on *Rutaceae*, particularly the more or less remote citrus relatives belonging to the subfamily *Citratae*, in the hope of obtaining more helpful information on the origin of the different forms of citrus scab, such as the sweet orange fruit scab referred to above and Australian citrus scab caused by *S. fawcettii scabiosa* Jenkins. The present study may be a beginning. Both *Hesperethusa* and *Clausena*, although rather remote relatives of *Citrus* that do not belong to the tribe *Citrinae*, are, nevertheless, closely enough related to *Citrus* to be grafted on it, as shown by Swingle.⁷—ANNA E. JENKINS, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

⁶ Jenkins, A. E., and H. S. Fawcett. Records of citrus scab family from herbarium specimens of the genus *Citrus* in England and the United States. *Phytopath.* 23, p. 476, fig. 1, A and B. 1933.

⁷ *Loc. cit.* See footnote c.

REPORT OF THE SUMMER MEETING OF THE AMERICAN
PHYTOPATHOLOGICAL SOCIETY HELD AT THE
UNIVERSITY FARM, ST. PAUL, MINN.,
JUNE 25-27, 1935

Through the efficient work of the local committee, headed by E. C. Stakman, a successful series of sessions and field trips was enjoyed by about 60 members and many visitors in connection with the summer meeting of the American Association for the Advancement of Science. No formal scientific papers were presented. An interesting symposium was held on the Past, Present and Future of Plant Pathology, at which H. L. Bolley entertainingly presented high lights from nearly fifty years of experience in this field of science, while E. M. Freeman, looking into the future, presented convincing arguments for the coordination of plant pathological research with related sciences, for the development of more team-work in attacking large or complex problems, and for the maintenance of the autonomous guild or scientific group organization in State or Federal agencies, if the most effective public service is to be rendered in research and regulatory work. Energetic round-table discussions on a wide range of topics at two sessions proved valuable. The Society participated in the joint symposium on Improving the Germ Plasm of Domestic Plants and Animals with Section O of the A. A. S., and affiliated societies.

The extensive field, laboratory, and greenhouse investigations at the Minnesota Experiment Station relating to plant diseases and breeding for resistance were inspected, together with an interesting exhibit of current research in the Department of Plant Pathology and Botany. Excursions were made into the region about the Twin Cities to observe plant-disease conditions in the truck, fruit, and ornamental plant-producing areas and at the Fruit Breeding Farm of the Experiment Station. Opportunities for personal conferences were abundant.

By vote of the members present a committee was appointed to work toward the development of a more comprehensive, coordinated program of potato improvement and another committee to work toward coordinated testing of seed-treatment methods. A resolution was passed emphasizing the necessity of adequate, fundamental biological preparation as a basis for satisfactory instruction in plant pathology, and deploring the tendency of administrators in some institutions to reduce the basic biological science requirements. Another resolution was passed to the effect that "The American Phytopathological Society desires to encourage efforts toward laying a sound foundation for an adequate classification of plant viruses. Recognizing that this can be attained only after a thorough study of the

viruses, their properties, their insect vectors, host ranges, their effects on the host plants, etc., the Society desires to encourage cooperation and coordination of effort toward this end."

At the enjoyable plant pathologists' dinner, a series of skits by the Minnesota members penetratingly interpreted the past, present, and future of plant pathology, and a loving cup was presented to the pioneer plant pathologist, Dr. H. L. Bolley.

HOWARD P. BARSS,
Secretary.

PHYTOPATHOLOGY—AND ITS FUTURE¹

E. M. FREEMAN²

The history of phytopathology's past is well known to this audience. I need not discuss it. The so-called present is merely the recent past. That leaves only the future for me to discuss.

In considering the future of plant pathology in the light and knowledge of the past, it seems to me highly important that we analyze at the outset the important concepts in our collective mind that it includes. To some the future problems revolve about investigational techniques, to others governmental organizations are paramount, and to still others the future personal fortunes of those engaged in this field are most absorbing.

Plant pathology (1) comprises a field of scientific knowledge and investigation; (2) it includes an application of knowledge and skill in the control of plant diseases; (3) in its socio-economic aspects it demands an organization not merely of the scientific facts and practices but of the social, economic, governmental, and professional forces and individuals that contribute to the field and (4) it houses a family of scientists, teachers, technicians, and practitioners that constitute a guild of plant pathologists. Perhaps there are other categories that merit inclusion, but these are outstanding to my mind, and their consideration will consume all of my time in this symposium.

The Science. As a science, plant pathology is inextricably interwoven with the other biological sciences and particularly with botany and its various other subsiences. Perhaps its twin sister may be said to be physiology. More probably botany, that prolific mother of plant sciences, may have given birth to quintuplets whose names were pathology, physiology, mycology, bacteriology, and morphology. They were born in that golden age of the renaissance of botanical science in the middle of the past century. The attending physicians became permanent and brilliant stars in their fields of science; Hofmeister, the interpreter and harmonizer of cryptogamic and phanerogamic morphology; the Tulasnes, discoverers of fungus versatility; Kühn, first to merit the degree of plant doctor; and DeBary, bacteriologist, morphologist, mycologist, pathologist, master mind and botanical savant. Nor must we overlook their colleague, Darwin, who modestly

¹ Read before the opening session of the Summer Meeting of The American Phytopathological Society, held at the University Farm, St. Paul, Minn., June 25-27, 1935.

² Chief of the Division of Plant Pathology and Botany, University Farm, St. Paul, Minn.

expounded the principle around which all of their infant sciences revolved and evolved.

Almost a century of scientific achievement has not altered that close relation of plant pathology with its sister sciences. Rather has the complexity of family relation increased. Ecology, cytology, biochemistry, genetics, biometry, and others have grown to youth or maturity and are contributors to, if not part and parcel of, the field of plant pathology. The enormous expansion and increasing complexity of all of these fields of plant science have steadily constricted the scope of operation of the individual scientist. A DeBary, superman in many fields, is no longer humanly possible, and it needs no divine power of prophecy to predict that he will never again appear. Plant pathology of the future must content itself with a growing intensity of specialization. Paradoxically coupled with this increasing specialization will be found an increasing demand for a knowledge of the literature of the sister and other sciences.

The realignment to these modern complexities has already taken place in the more recent past, and there can be little doubt of the path for the future. Plant pathology must coordinate its research more and more with those sister sciences. The superman must be succeeded by intelligent, generous, genuine, and spontaneous cooperation. Team-work actuated by desire and necessity is the only effective substitute. Because this may be difficult, irritating, or inconvenient is no valid argument against it. Modern complexity in science as well as in social, economic, and political fields demands and must have this increasingly complex organization. The individual who clings to the extreme raw individualism of the scientific past must be content to operate in an increasingly restricted orbit or disappear entirely as an important contributor of knowledge.

I need not elaborate greatly on what that cooperation involves. It means that investigations such as genetics of plant pathogens, biochemical interpretations of disease manifestations, cytological explanations of life-history processes, biometrical studies of pathological phenomena, morphological investigations of host and pathogen are subjects of study for pairs or groups of scientists professionally trained in diverse fields. To the individual plant pathologist it means the careful selection of his particular field of research and study and a cooperating knowledge of those sciences that impinge on his field so that he may intelligently seek and effectively use the aid of specialists in those fields.

One may properly ask: Are we doomed to become workers in a teeming anthill, each with his little load of assigned and tedious labor? I think not. Specialization can best be enriched by a broad knowledge of surrounding territory. The sacrifice of time and effort in going even far afield to become acquainted with the work, the attitudes, the techniques, the successes and

failures in related and even unrelated fields are enlightening and stimulating experiences for every scientist.

The Practice of Plant-disease Control. In the practice of plant-disease control I find myself constantly on guard against unjustified analogies with the practice of medicine among humans and other domesticated animals. These analogies are alluring because of the long history of human medicine and the outstanding achievement of that great group of sciences which underlie it. To my mind the practice of plant disease control is so circumscribed by different conditions that conclusions from any analogies must be scrutinized with the greatest of care.

It is quite obvious at the outset that the practice of human medicine concerns only one biological host species on which centuries of research have builded an incalculable fund of knowledge and in which an interpretation of the responses to treatment are greatly aided by human intelligence. In plant pathology the number of host species is legion. The disparity is further accentuated by the multiplicity of pathogens on each plant host and the modern discoveries in biological forms of pathogens do not at all simplify the matter. Furthermore, the practice of disease control is inextricably bound up with cultural practice, soil conditions, climatic factors, and all of these may vary with each host species. And, finally, many of the host plants may be of such low economic value that the cost of disease control would be prohibitive, while human life is said to be priceless.

The plants that may be said to be of economic significance are not only imposing in the vast number of species, but they are nothing less than bewildering in their diversity. They range from mushrooms to giant trees, from inhabitants of the moist tropical jungle to cacti of the desert; they include producers of foodstuffs in all climates, soils, altitudes, and latitudes; they range from thallophytes to composites.

The great and fundamental difficulty of the application or practice of the several plant science specialties, including plant pathology, in the art of plant production, lies in the basic difference of the vocations involved. The scientist's specialty must necessarily follow some path in the field of his science. He may choose genetics, pathology, or some other special field, but he cannot cover all or many of them.

In sharp contrast to this, the plant grower must confine his efforts to one or a limited number of crop plants. His business never coincides with the activities of one scientific field. He must apply the results obtained by specialists in soils, genetics, plant pathology, and all the other sciences.

Of course, it is conceivable that a new type of trained specialist, such as a plant doctor, eventually may evolve—one who has equipped himself primarily with a knowledge of the pathology of a single crop or a small group of crop plants. Perhaps such a profession may succeed financially as well

as professionally. One shudders to think, however, of the quacks and crooks who will pose as experts in that day. One is reminded of the fake medical cults of today and the difficulties encountered even in controlling them, to say nothing of their extermination. Indeed, we already have a vocation (or is it a profession?) of tree surgery, which has rendered some real service, but in whose name innumerable biological crimes have been committed and to whose coffers has flowed much money extracted from a gullible public.

If we take stock of the status of the application of plant pathology today, we find, perhaps, the closest analogy to human medicine in socialized medicine, the goal of many medical educators. Our agencies of plant-disease control are largely governmental through educational and research institutions. Advice and information in every phase of crop production cost only the effort of asking for them. That service includes the experience of the best specialists in the whole gamut of plant sciences. But the extent and nature of that service are sadly inadequate for the task, even though the state and federal support may be considered generally generous. Perhaps the greatest weakness in our present clinical system lies in the lack of diagnoses in the field and the ignorance of the grower as to his specific needs and the means of supplying them.

To sum up this matter of the future of the practice of plant-disease control, two developments seem probable. Certainly the established clinical methods of experiment stations and extension departments, federal departments, and other government agencies will continue to combine their research activities with the practice of control as in the past. Since commercial specialists in such control have already appeared in our economic order, it seems fair to assume that they will continue. The more probable development of such service seems to be through farm management and similar advisory service corporations large enough to include specially trained men in the several fields basic to the crops concerned. Perhaps the general practitioner or plant doctor may also find a place.

The Organization of Plant Pathology. Research, teaching, and extension of knowledge concerning plant pathology are centered almost exclusively in State or Federal institutions of research and education. Its coordination with other plant and animal sciences in the various governmental and educational institutions is not merely an academic question but one of vital importance. Like every other plant science, it merits prominence and support commensurate with its potential and demonstrated value to agriculture and the general public. Plant pathologists have abundantly demonstrated the value of their investigations and services. Even examples are unnecessary to this audience. Neither is any proof needed of the potential values of this specialized science, which will surely grow with the increasing com-

plexities of our agricultural and economic progress. But the development of any science may be seriously affected by its position in the educational or governmental institution where it functions.

While most large State-supported universities and agricultural colleges maintain separate departments of plant pathology, their subordination, in some institutions, to other coordinate scientific fields seems to me unwise and, for the most part, unjustifiable. Plant pathology has important activities beyond the confines of either horticulture or agronomy. Its subordination to other sciences in smaller institutions or organizations may be unavoidable because of insufficient revenue, but such necessary administrative adjustment should, by no means, restrict the broader activities of the plant pathology staff.

Of greater concern, however, is the administrative development which appears from time to time in State and Federal so-called reorganizations. The organization and coordination of institutional departments and bureaus are not simple matters. There are fundamental difficulties which necessarily give rise to conflicting interests. An analysis of what I have in mind may well begin with the organization in a large university or agricultural college. Since teaching and research are the primary functions of the staff and since extension service is frequently segregated into a separate unit, it follows almost inevitably that the departmental organization follows the lines of scientific specialization. Departments consist of groups of specialists trained in the same professional guild. New departments arise as some subspecialty attains sufficient importance and prominence to merit an independent and autonomous administrative status.

To my mind the important feature is not so much the catalog status of any given science. The paramount concern should be the best opportunity of guilds of similarly trained scientists with common educational and research objectives to work together to their best advantage. No administrative scheme is worth more than the paper on which it is written if it does not have an understanding and sympathetic administration in charge. No administrative scheme can possibly eliminate the overlapping of sciences and departments. From their very nature they must overlap. It is the chief business of the administrator to see that these overlapping borders are well oiled to prevent excessive friction. Too often the administrator assumes that a reorganization or a dictatorial ukase will eliminate these problems, whereas the chief need is an oil can with a liberal supply of cooperation—spontaneous if possible, compulsory if necessary—and not one drop of isolation in its mixture. While college and experiment station coordinations affecting plant pathology are not without their problems, these seem to me solvable on the basis of our present organizations which, in general, are fundamentally sound.

Of far greater concern, however, are government and State organization affecting the activities and status of plant pathology. Our Federal and State departments of agriculture are basically research or service organizations or both.

The professional guild basis, though historically entrenched here and there, is constantly confronted with the competition of a project system or a crop system of organization. I am not one to claim a sufficient wisdom to criticize such organizations as universally inappropriate or inefficient. But all these bureaus and departments are fundamentally grounded in the sciences which solve their problems. Whatever may be the administrative device used to accomplish the necessary ends, two things stand out in my mind as fundamental. First the recognized professional guilds must be maintained in sufficiently large groups to furnish conditions under which they may cooperate and maintain their guild existence. Plant pathology is one of these recognized groups. An organization that subordinates whole professional guilds to a single crop plant or commodity project may thereby demote professional scientists to skilled labor functions and may so delimit the scope, activity, and service of such scientists that inexcusable duplication is encouraged and time and money-saving cooperation discouraged. It is basic to the nature of governmental service in agriculture that the horizontal demand for service in all fields of science is crossed at right angles by the scientific guilds that render that service. No organization devised by man can possibly change that fact. The solution lies not in cutting either the horizontal or the vertical lines. It lies in the administrator's skill and responsibility to see that both lines are kept intact with the least possible confusion and friction. It is his business, for which there can be no substitute scheme of organization, to coordinate in a horizontal plane the activities of all of the scientific guilds to the use of the growers of one crop plant. It is equally his business in the interest of public welfare and economy to maintain the efficiency, autonomy, and cooperative facilities of each of the important scientific guilds that give their service to that crop plant or to related crop plants. Even in a broader field it seems to me this principle ought to apply. Repeated attempts have been made to remove the United States Forest Service from the Department of Agriculture to the Department of the Interior. Such removal would be a wholesale separation of the numerous scientific guilds that serve forestry from their close contacts with the closely allied guilds of the field of agriculture of which they are an integral part. The recent removal of the Soil Erosion office to the Department of Agriculture seems to be at least a second-thought recognition of what is obviously the sound coordination of that work.

Of all the problems that confront plant pathology, one of the most difficult and perplexing is that of the quarantine and regulatory control

of plant diseases and insect pests. To my mind this problem offers fundamental difficulties that are not always appreciated. While I am firmly convinced that the principles of organization stated above should apply wherever possible, there are other factors that cannot be ignored. Chief among these factors is the legal nature of the enterprise. Quarantine and regulation are neither research nor education, though both of the latter may be valuable aids. They are laws and require enforcement. A new guild enters the picture. Indeed, the enforcement of the law is the primary objective and would seem to demand a preferred position in organization for administration. It is obvious that in the Federal control, where the Department of Agriculture has been faced with regulatory activities in almost every field of agriculture, that the administration of large groups of regulatory activities be concentrated. After all, regulatory work may be a distinct detriment when coupled too closely with publicly supported research and educational institutions.

But there is another side of this problem that is of particular interest to the future of plant pathology. Plant quarantines and regulatory acts frequently involve plant diseases as well as insect pests. Regulatory administration must have the aid and assistance, if not the actual direction, of trained scientists. The importance of plant diseases in the regulatory field is sufficiently large to justify a greater participation from the guild of plant pathologists. Regulatory administrations, I believe, increase their efficiency by observing the principles of guild autonomy discussed above. It is to that end that pathologists should aim, both in the interests of their own science and for the improvement of public service.

The Guild of Plant Pathologists. Plant pathology has merited and won recognition as a plant science throughout the world. Its importance in the future cannot diminish but must increase. With the inevitable and increasing complexity of agricultural industries will come more and greater specialization within the guild. But that specialization involves not so much a narrowing of training within the science itself as an intelligent coordination with sister plant sciences. New fields of professional activity have already opened up. Many more will follow. Their number and importance will depend largely on the initiative of the future members of the guild. Success will depend on their understanding not merely of the scientific facts involved, but also on a grasp of economic conditions and currents. It will require the application of scientific training to public and private enterprises. It will need scientific imagination coupled with practical knowledge and common sense. It will offer a career in fundamental research, pure and undefiled or gloriously practical. It will offer a satisfying field of combat against one of the most potent, destructive forces of nature.

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Progress in the Development of a New Tomato Variety Resistant to Leaf Mold. L. J. ALEXANDER.

The inability to secure either parental fruit type from crosses between individuals of the small-fruit homozygous resistant lines and individuals of the large-fruit homozygous susceptible variety, Globe, necessitated a long program of backcrossing and selection. At the present time a number of selections, still heterozygous for resistance, produce good quality fruit that appear equal in size to that of Globe and show promise of high yield. F_1 individuals resulting from crosses between homozygous resistant and homozygous susceptible parents are all resistant. Individuals of the F_2 progenies segregate for resistance into a ratio closely approaching that of 3 resistant to 1 susceptible. Progeny tests were made from 82 of these F_2 plants. These yielded 19 homozygous resistant, 42 heterozygous resistant, and 21 homozygous susceptible progenies. F_1 individuals resulting from crosses between heterozygous resistant and homozygous susceptible parents segregate for resistance into a ratio closely approaching that of 1 resistant to 1 susceptible. Progeny tests were made from 122 of these F_1 plants. Fifty-nine of the progenies were heterozygous for resistance and 63 were homozygous for susceptibility.

Growth and Distribution of Ceratostomella ulmi in Tissues of Elm. W. M. BANFIELD and A. L. SMITH.

Spores and a sparse mycelium are found in the discolored vessels of elms affected by the Dutch elm disease. Conidia are produced by the mycelium in the vessels, are distributed by the sap stream, multiply by yeast-like budding, or give rise to new hyphae. The hyphae grow through bordered pits into contiguous vessels and there release other conidia. The vascular system of the current season's growth sheath may be quickly invaded throughout all parts of the diseased tree. The mycelium in necrotic tissues mostly parallels the vertical axis of the cell lumina occupied. In sterilized inoculated wood it develops more abundantly in the ray parenchyma cells, the lumina of which commonly are choked thereby. Hyphae penetrate along the rays from ring to ring in the wood of root and stem in necrotic tissue. Hyphal strands commonly penetrate cell walls *via* bordered pits in such tissue, but usually penetrate directly by means of very constricted mycelial strands. Conidia are copiously produced in the lumina of many fibre, vessel, or ray parenchyma cells in sterilized inoculated wood. Yeast-like multiplication of the conidia chokes the lumina of some cells with spores. (Emergency Conservation Work and Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.)

Correlation of Pathogenicity and "Viscosity" in Cultures of Phytomonas tumefaciens. T. O. BERGE, A. J. RIKER, and I. L. BALDWIN.

Differences in ring pull have been detected with the duNoüy tensiometer in giant colonies of single-cell sister cultures of *Phytomonas tumefaciens*. One of these cultures is pathogenic to tomato at 27° but not at 32° C.; the other is nonpathogenic on this host. Less pull is required to separate the ring from the surface of colonies of the pathogenic strain than from colonies of the nonpathogenic strain when grown at 26° C. on various media, respectively; *e.g.*, yeast-extract, bean-extract, carrot-extract, asparagine, and nitrate agar. More ring pull for both cultures was required when they were grown at 32° C. Also in carrot-extract, tomato-extract, and bean-extract media, nonpathogenic cultures have been found to show a higher viscosity in a modified Saybolt viscosimeter than parallel pathogenic cultures. Since such differences in "viscosity" appear to be associated with the character of the bacterial gums produced by the cultures, it seems that these substances deserve attention in relation to pathogenicity.

Some Insect and Host Relationships of the Potato Yellow Dwarf Virus. L. M. BLACK.

The clover leaf hopper, *Aceratagallia sanguinolenta*, has been found to be a vector of the virus, and viruliferous insects have been collected from clover fields. Medium red clover, *Trifolium pratense*, is susceptible to the virus, and is the chief host plant of the clover leaf hopper. The leaf hoppers become able to transmit the virus about 9 days after feeding on diseased plants and are capable of harboring the virus from early November until early April. The present-known geographical range of the insect is much wider than that in which the disease is prevalent. Koch's report of transmission by *Myzus persicae* was not confirmed, although the same strain of aphid was employed. Muncie has reported transmission by *Empoasca fabae* and *Macrosiphum solanifolii*. Results with these insects and several others have been negative. The difference in vectors offers a good explanation of the contrasting field behavior of yellow dwarf, on the one hand, and leaf roll and mosaic on the other.

Two Types of Yellows Resistance in Wisconsin All Seasons Cabbage. L. M. BLANK.

Resistance in cabbage to *Fusarium conglutinans* consists of at least two types. Type A is inherited as a single dominant character. Type B is more complex genetically. In Wisconsin All Seasons single-plant lines containing either type A or type B have been established and carried through 3 generations by self pollination. In field trials, progenies containing type B vary in the amount of resistance displayed, ranging up to complete freedom from disease symptoms. In greenhouse trials over a range of soil temperatures, the 2 types of resistance may be readily distinguished. Type A remains stable over a wide range of controlled soil temperatures. Type B resistance varies in degree in different progenies and breaks down with increase in soil temperature until it is usually completely suppressed at about 24° C. At moderate greenhouse temperatures some lines carrying type B resistance show a high percentage of diseased, but mildly affected, plants. Other lines, which are fairly high in resistance in the field, show a high percentage of severely diseased plants in the greenhouse. Type B resistance is transmitted to and expressed to a certain degree in F₁ hybrids from crosses with homozygous-susceptible plants. (Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, and the University of Wisconsin.)

Field Control of Die Back and Black Spot. G. T. BOYD and J. J. TAUBENHAUS.

Fair control of *Diplodia* die back of roses was obtained by cutting off at weekly intervals all the flower buds from the plants. Better control was obtained when, in addition to the weekly disbuddings, the plants were either sprayed with Bordeaux mixture or dusted with a mixture of 10 parts of 300-mesh sulphur, 1 part monohydrated copper sulphate, and 1 part Paris Green. Neither spraying nor dusting, without disbudding, was an effective control measure. Slight control of black spot was obtained by weekly removal of flower buds. This was due probably to the greater vigor of the disbudded plants. Good control of black spot was obtained when the plants were disbudded and also sprayed with Bordeaux mixture or dusted with the sulphur mixture mentioned above.

The Effect of Certain Chemicals on the Defoliation of Rose Plants.—G. T. BOYD AND J. J. TAUBENHAUS.

Various diseases and decays frequently develop on bundled and winter-stored rose plants that fail to shed their leaves during the harvesting season. Preliminary tests were carried out in 1935 with a large number of spray materials for defoliation of such plants. Commercial lime sulphur when diluted 1:8 with water or when used with 0.25 per cent H₂SO₄ caused severe leaf burn but no defoliation. Dilutions of arsenic pentoxide alone or in combination with H₂SO₄ caused severe injury to both foliage

and canes. Various dilutions of H_2SO_4 and $CaSO_4$ or $FeSO_4$ were more promising as defoliating agents. Prompt defoliation was secured on Paul's Scarlet Climber and President Hoover with a spray consisting of 2 lb. of either $CaSO_4$ or $FeSO_4$ in 50 gal. of a 0.25% solution of H_2SO_4 . With the Talisman variety only partial defoliation resulted from the use of these materials. A commercial sticker (Aresket) was used in these combinations to assure complete coverage of foliage.

Inflorescence Blight of the Date Palm.—J. G. BROWN AND KARL D. BUTLER.

A fungus blight of the inflorescence of *Iteema*, *Maktoom*, *Sayer*, *Khiri*, and seedling date palms has caused some loss in Arizona. Symptoms include water soaking and subsequent discoloration of infected parts, death of flowers, and fracture of the main stalk, which becomes so weakened that it cannot bear the increasing weight of the fruit in the case of the pistillate inflorescence. From infected, surface-sterilized tissues 2 *Fusarium* spp. were isolated: *F. moniliforme*, identified by two *Fusarium* specialists, rapidly decayed healthy date rachi into which it was inoculated and from which it was afterward reisolated; the other species, identified by one authority as *F. semitectum*, by the other as *F. lateritium* var. *fructigenum*, caused an almost identical blight, which, however, was slower in its action.

Seed Treatment As a Control for Damping off of Alfalfa And Other Legumes.—WALTER F. BUCHHOLTZ.

Seedling stands of alfalfa, white sweet clover, Dalea, Lespedeza, red clover, and alsike clover were improved by treating the seed with an organic mercury dust before planting in *Pythium*-infested soil in the greenhouse. Only small increases were obtained with alsike and red clover, and white Dutch clover did not damp off in the soil used. In the field, emerged seedling stands of alfalfa were improved 8 to 145%, depending on the degree of soil infestation. On one area, apparently nearly free of *Pythium*, alfalfa seedling stands were depressed 5 and 11% by seed treatment. Counts taken from a field stand of alfalfa grown on unlimed Clinton silt loam from treated seed were 50% higher than those taken from an adjacent limed area seeded with inoculated seed. Nodules were found on the roots of plants grown from both treated and inoculated seed.

The Occurrence of Pythiaceae Parasites at Different Soil Levels in Relation to Fallowing Practices.—WALTER F. BUCHHOLTZ.

The *Pythiaceae* fungi, parasitic on alfalfa seedlings, have been found most abundantly in the upper 3 to 5 inches of top soil of Webster silt loam, Clarion loam, and Tama silt loam. An area of Webster silt loam that had been fallow two years produced a 39% emerged stand¹ of alfalfa seedlings, while an adjacent area that had grown a crop of corn the year before and had been spring-plowed, yielded a 75% stand. When the top two and the next two inches of soil were interchanged, the fallow Webster silt loam produced an 80% stand.

Melanconium betulinum on *Betula* in Illinois.—J. C. CARTER.

Apparently because their vigor was greatly reduced by the drought of 1934, birches (*Betula alba* and *B. papyrifera*) have been attacked this year, in both nurseries and ornamental plantings, in widely separated sections of Illinois, by a die-back disease with which a *Melanconium* is uniformly associated. It appears identical with *M. betulinum* as represented by No. 1998 of Sydow's *Mycotheca Germanica*. Fruiting bodies

¹ Percentage stand is calculated on the basis of the average number of seedlings obtained from a similar planting on nearly *Pythium*-free soil.

first show as small raised places in the cortex and develop into white-tipped, stromatic acervuli. Conidia germinated freely on Difco plain, malt-extract, potato-dextrose, and corn-meal agars. However, germ tubes did not develop beyond a length of $110\ \mu$ on plain agar but on other agars good colonies developed. This fungus grows slowly in culture. On malt-extract agar growth averaged 13 mm. in 48 hours and 25 mm. in 96 hours; on potato-dextrose agar 14 mm. in 48 hours and 32 mm. in 96 hours; and on corn-meal agar 13.5 mm. in 48 hours and 27.5 mm. in 96 hours. Of 3,000 counted spores suspended in water, 34.6% had germinated after 24 hours and their germ tubes averaged $33.1\ \mu$ in length. After 48 hours 50.2% had germinated and the germ tube length averaged $35.5\ \mu$.

Does Heterocaryosis Account for the Production of Variants in Helminthosporium?—

J. J. CHRISTENSEN AND F. R. DAVIES.

Variants occur frequently in *Helminthosporium*. The hyphal cells, conidia, and germ tubes are multinucleate, and hyphal anastomoses between races of *Helminthosporium sativum* and other dark-spore species are common. Cultural comparisons were made of 468 conidial isolates from 154 individual conidiophores obtained from mixtures of 2 distinct races or species growing on agar drops or from barley tissue infected with 2 or more distinct races or species. All isolates from the same conidiophore, with two exceptions, were identical culturally. Two hundred sixty eight monosporous isolates selected at random from mixed colonies on agar drops resembled the parental types. From 2 to 5 hyphal tips were isolated from each of 63 germinating conidia taken from colonies giving rise to variants or from mixed colonies on agar drops. With one exception, the isolates from a single conidium were identical with the original culture. Young conidiophores are uninucleate; consequently, all nuclei in conidia produced on the same conidiophore are derivatives of a single nucleus. The results indicate that the variation in the species studied is due primarily to nuclear change rather than to heterocaryosis.

Studies on Methods for the Measurement of Disease Resistance in N. tabacum.—E. E. CLAYTON.

Extensive collections of tobacco varieties and strains are being studied for resistance to black rootrot (*Thielaviopsis basicola*), Granville wilt (*B. solanacearum*), Fusarium wilt (*Fusarium oxysporum* var. *nicotianae*), black shank (*Phytophthora parasitica* var. *nicotianae*), stem rot (*Sclerotium rolfsii*), mildew (*Peronospora tabacina*), wild fire (*B. tabacum*), black fire (*B. angulatum*) and mosaic. It has been found that the method of testing must give consideration to the type of resistance and factors that modify its expression. Inoculation technique is important with Granville wilt because both morphological and physiological resistance exist. Age of plants is an important factor in studying resistance to mildew and wild fire. Temperature conditions by favoring or retarding disease development may reveal or obscure difference in degree of resistance. Plan nutrition materially affects resistance to black fire and wild fire, and with these diseases storm effects must also be considered. By carefully conducted tests in laboratory and greenhouse it is possible to confine field work to small selected groups, with a material saving in time and expense.

Cross Inoculation and Morphological Studies on the Peronospora Species Occurring on Chenopodium album and Spinacia oleracea.—HAROLD T. COOK.

Cross inoculations and morphological studies were conducted with the *Peronospora* sp. occurring on *Chenopodium album* (lamb's quarters) and *Spinacia oleracea* (spinach) in Virginia to determine if they are identical. In the cross-inoculation tests lamb's

quarters plants were infected only by conidia from this host, and spinach plants were infected only by conidia from spinach. Morphological studies showed that the conidia, conidiophores, and oospores of the *Peronospora* on spinach differed from those of the *Peronospora* on lamb's quarters. It is concluded from this study that the 2 are distinct species and that *C. album* does not serve as a host of the spinach mildew fungus.

Comparison of the Effectiveness of Seed-treatment Materials for the Prevention of Seed and Seedling Decays in Eastern Virginia.—HAROLD T. COOK AND JOHN A. CALLENBACH.

A number of seed-treatment materials were compared for their effectiveness in the prevention of seed and seedling decays of tomatoes under greenhouse and field conditions. These treatments were supplementary to HgCl_2 treatment (1:2000 for 7 minutes) for the control of seed-borne diseases. ZnO , Cu_2O , Vasco 4, and Semesan were applied as dusts and tested both in the greenhouse and field. CuSO_4 , applied as a soak, was tested only in the field and resulted in severe injury. Vasco 4, ZnO , Cu_2O , and Semesan ranked in the order named in both greenhouse and field tests.

A Mosaic Disease of Tithonia rotundifolia.—MELVILLE T. COOK.

A single specimen of *Tithonia rotundifolia*, an introduced ornamental plant in Puerto Rico, was found showing large yellow blotches. Inoculations were made to other plants and the symptoms reproduced. Inoculations of it to tobacco and of the yellow tobacco mosaic to *Tithonia* gave negative results. Only one other record of a virus disease on this genus has come to the attention of the writer. That was by van der Bijl of South Africa, who reported a virus disease on *T. diversifolia* in 1931. However, *Tagetes* is a synonym of *Tithonia*; and several virus diseases have been reported on *Tagetes erecta* and *T. patula* in the United States.

Phloem Necrosis in the Stripe Disease of Corn.—MELVILLE T. COOK.

This is the third record of phloem necrosis, which is transmitted in the seed and is not abundant in Puerto Rico, probably because it causes sterility in many diseased plants. Histological studies showed phloem necrosis in every case and a necrosis in the parenchyma in a few cases. In the most severe cases there was a complete breakdown of a large number of cells. In some cases there was an increase in the number of fibrous cells. The chloroplasts in the regions of necrosis are reduced in number and size. There is no evidence of disintegration, but abundant evidence of inhibition of these chloroplasts.

Some Principles Underlying the Fungicidal Action of Mercury in Soils.—ROBERT H. DAINES.

In soils where mercurials are effective as fungicides, mercury compounds are reduced by the soil to metallic mercury, which migrates in the soil as mercury vapors. Any factor that prevents the conversion of a mercury salt to metallic mercury destroys the fungicidal effects of the mercurial. Some of these limiting factors are: The presence of mercury-precipitating ions; a soil with a high mercury-binding capacity and a soil having a high oxidation, and, conversely, a low reducing potential. Mercurials consisting of metallic mercury deposited on suitable carriers were found to give more satisfactory control of *Rhizoctonia* stem lesions than any of the standard mercurials when used either as a seed potato dip or soil treatment. Mercury on Bentonite was found to give excellent control in some soils, but only partial control in others. This reduction in effectiveness was found to be corrected by using amalgams of metals above mercury in the electromotive series. In the presence of the other metals the oxidation of metallic mercury is delayed and its effective period prolonged.

Corn Smut—Latent and Expressed.—GLEN N. DAVIS.

In the fall of 1934 and 1935 the leaf sheaths were stripped from corn plants exposed to natural and artificial smut infection. Small smut galls, not large enough to rupture the leaf sheaths, aggregating 39.1 and 30.7%, respectively, of the total infection, were found at the nodes. Smut mycelium may remain latent in the nodal buds for a long period resulting in late appearance of nodal infections. Field and laboratory experiments to determine if such might be the case, including 5 years' results on inoculated and noninoculated plants injured to stimulate axillary bud development show, when figured on a per-plant basis that in the noninoculated series the removal of the ears gave an increase of 24.6%, and removal of tops and ears increased by 45.1% in the number of nodal smut galls over that in the checks. In the inoculated series, removal of the ears gave an increase of 17.2% and removal of the tops an increase of 81.7% in the number of nodal galls over that in the inoculated noninjured checks. Laboratory examination of 262 axillary buds from 50 inoculated sweet-corn plants, variety Golden Bantam, at intervals of 14-67 days after inoculation showed 140 to be infected with smut.

The Occurrence in the United States of Two Types of Teliospores of Tranzschelia pruni-spinosae.—JOHN C. DUNEGAN.

Teliospores of *Tranzschelia pruni-spinosae* produced on peach leaves differ morphologically from those produced on indigenous species of the genus *Prunus*. The two types of teliospores are identical with the *forma discolor* and *forma typica*, respectively, previously described in Europe, but hitherto not recognized in the United States. Inoculation experiments show that the *discolor* type, occurring on the peach and other cultivated hosts, is associated with an aecial stage on *Anemone coronaria*, while the *typica* type, occurring on indigenous hosts, is associated with an aecial stage on native species of *Hepatica* and *Anemone*. Cross-inoculation experiments and other studies indicate that the two types of teliospores represent two forms of the rust. These two forms have different aecial host plants, the urediospores, though similar do not cross infect, and the teliospores are readily distinguishable. They probably represent distinct, though closely related, species but the final decision on this point is reserved until the host relations of the basidiospores have been investigated.

Bacterial Wilt of Potatoes, Tomatoes, and Eggplant Controlled with Sulphur and Limestone.—A. H. EDDINS.

Bacterial wilt (*Bacterium solanacearum*) was commercially controlled (between crops in 1935) in experimental plots of potatoes, tomatoes, and eggplant at West Toccoi, Florida, by applying sulphur to the soil in June, 1934, then following with limestone in November. The causal organism was killed or rendered nonvirulent by broadcasting 800 lb. of freshly inoculated sulphur per A., which reduced the soil reaction from pH 5 to slightly below pH 4. The soil reaction was then restored to approximately the original pH by broadcasting 3,000 lb. of dolomitic limestone per A., which induced normal growth and yield. This treatment gave an increase of 23.1 bbl. (63.5 bus.) per A. of healthy marketable potatoes of the Spaulding Rose Variety. Only 0.8% of the tubers in the treated plots were infected; while 70.4% were infected in the controls. Tomatoes yielded 1 lb. of marketable fruit per plant in the treated soil with 17.9% of the plants killed; in nontreated soil the yield was only 1.0 lb. of marketable fruit per plant with 98.8% of the plants killed. Eggplants yielded 5.2 lb. of marketable fruit per plant with 5.9% of the plants killed in the treated soil; in nontreated soil, the yield was only 0.4 lb. per plant with 70.6% of the plants killed.

Pythium Root Rot of Milo. CHARLOTTE ELLIOTT, L. E. MELCHERS, C. L. LEFEBVRE, AND F. A. WAGNER.

For the past 10 years milos in certain localities of the Southwest have been subject to a root rot that has increased in severity on infested soil until it is no longer possible to produce a crop from susceptible varieties. Diseased plants are stunted and when 10-12 inches high the leaves begin to turn yellow along their margins and at the tips. This yellowing progresses until the plant dries without heading. All of the fine roots are destroyed and the larger roots and interior of the crown show a dark red discoloration. Soil treatments with steam, formalin, or acetic acid have shown that the causal organism is soil-borne. Isolations from diseased roots have given a fungus that, on inoculation under greenhouse conditions, has produced the same symptoms as those occurring on plants grown in naturally infested soil. This fungus has been identified as *Pythium arrhenomanes* the causal organism of a root rot of corn. Certain strains and hybrids of milo and Darso are susceptible to the disease. The kafirs and sorgos tested are resistant or immune. Resistant strains of susceptible milos have been developed at the Garden City station and tested there for several years and at other stations in the Southwest. (Cooperative investigations between the Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

The Fungi Causing Decay of Pears in Washington.—HARLEY ENGLISH AND F. D. HEALD.

Representative lots of pears from the various orchard sections of the state have been studied for the development of decay. From the large number of isolations made, 44 fungi, culturally different, have been obtained. Blue molds and gray molds were most frequently isolated. The different isolates are being studied to determine the species and their degree of pathogenicity. The rate of decay produced by the several isolates has been tested at room temperature (68-75°F.), common storage (40-50°F.) and at cold storage (32-35°F.). Some of the isolates caused decay at the three temperatures tested, others only at the higher temperatures, while a few were contaminants that caused no decay when pure cultures were used for inoculation. The two common forms, blue mold (*Penicillium* sp.) and gray mold (*Botrytis* sp.) were used in a series of inoculations of D'Anjou and Winter Nelis held at both common and cold-storage temperatures. Gray mold caused a more rapid decay at both temperatures than blue mold, and the D'Anjou pears were more rapidly decayed than the Winter Nelis.

Further Study on the Nature of Immunity of Monocotyledonous Plants to Phymatotrichum Root Rot.—WALTER N. EZEKIEL, J. J. TAUBENHAUS, AND J. F. FUDGE.

Previous work has indicated that juices expressed from the underground portions of monocotyledonous plants that are immune from *Phymatotrichum omnivorum* root rot, contain materials that, when added to synthetic nutrient solutions, inhibit growth of the fungus. Ether extracts of such juices are invariably inhibitory. Ethyl-ether extracts are more potent than petroleum-ether extracts. The ether-soluble potent material is not precipitated by acetone. It is removed from ether solution by aqueous sodium-carbonate solution, from which it has been recovered by ether extraction after acidification. Purified fractions prepared in this way completely prevent growth of *P. omnivorum* when added to nutrient solutions in amounts which supply often less than 0.05% of plant material. Such fractions have been prepared from onion bulbs, Gladiolus corms, and cane, canna, and Hemerocallis roots. Similar fractions of juices from susceptible, dicotyledonous sweet potato, carrot, and beet roots contained lower dry weights of material and did not impede growth of *P. omnivorum*. It seems probable that immunity of monocotyledonous plants

from root rot is due, at least in part, to relatively high concentration in roots of these plants of specific ether-soluble materials of an acidic nature.

Verticillium a Probable Cause of a Spinach Wilt in Western New York......E. L. FELIX.

A spinach wilt, characterized by yellowing, wilting, and interior discoloration of the vascular system, has been observed annually during the past 4 years in the Elba muck section of New York. Losses ranged from a trace to 10%, with an average loss of less than 1%. The constant association of a fungus agreeing in morphology and temperature relations with *Verticillium albo-atrum* and the absence of *Fusarium* or other organisms in the roots of recently infected plants suggest the probable pathogenicity of *Verticillium* for spinach. Several series of greenhouse inoculations in 1933 failed to produce the disease, but conditions may not have been favorable for infection. The optimum temperature for growth of the fungus on potato-dextrose agar was between 24° and 30° C. and the maximum between 30° and 35° C.

Effect of Take-all Lesions on the Roots, Crowns, and Culms of Wheat Plants.—HURLEY FELLOWS.

For five years observations were made on the interrelation of different degrees of severity of lesions on the roots, crowns, and culms of greenhouse cultures of wheat attacked by *Ophiobolus graminis*. A definite relation was found between the presence and severity of lesions on the organs under consideration. If one organ alone was diseased, it usually was the roots. Root infection was followed quickly by crown infection. Increases in the percentages of crown infection were accompanied: (1) by increases in root and culm infections, in the severity of lesions on all parts, in the loss of roots, and in the percentage of plants dead, and (2) by decreases in the average height of plants, in the percentage of culms that headed, and in the percentage of plants that tillered. All plants killed by *O. graminis* and those on which sporulation was observed were infected in roots, crowns, and culms. (Cooperative investigations between the Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Nitrogen Metabolism of Ophiobolus graminis. HURLEY FELLOWS.

Ophiobolus graminis, the parasite causing take all of wheat, has been found to be specific in its nitrogen requirements when the nitrogen was supplied in a modified Czapek's nutrient solution. Egg albumen, casein, peptone, and nucleic acid were the only compounds that were utilized by the fungus. The nitrogen of the other materials tried, both organic and inorganic, was unavailable. This unavailability was not affected by the hydrogen-ion concentration of the medium, by the source of carbon, nor by the presence of organic or inorganic growth-promoting materials. Many plant decoctions, as well as the tissue of several plants, were favorable media for *O. graminis*. (Cooperative investigations between the Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Browning Disease of Flax in the United States. H. H. FLOR.

Prior to 1932, *Polyspora lini*, the browning disease of flax, was known to occur in the United States only in Michigan. During the last three years it has been found in Iowa, Minnesota, North Dakota, and Oregon. A disease survey of flax fields in southern Minnesota and northern Iowa was made in the late spring of 1935. The stem-canker phase of the disease was found in 23 per cent of the fields inspected, and the percentage of plants attacked ranged from a trace to 15. The attacked plants were usually either

killed or severely stunted. Browning was found in widely scattered fields throughout the eastern half of North Dakota during the summer of 1935, but appeared to be causing but little damage. Field and greenhouse tests conducted at Fargo, N. Dak., have shown Bison to be the most susceptible of the commercial seed-flax varieties, followed in order of decreasing susceptibility by Red Wing, Linota, Buda, and Rio. The yield of Bison flax from browning-inoculated plots was reduced to 61% and the average weight per seed to 85% of that from noninoculated plots. Differences in oil content (percentage) and quality (iodine number) of seed from the severely diseased plants and that from normal plants were insignificant. (Cooperative investigations between the North Dakota Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Mosaic of Lima Beans. L. L. HARTER.

Mosaic of Lima beans has been reported only a few times in the United States, and in no case was there indicated any considerable amount of damage to the plants or reduction in yield. During the summer of 1935, mosaic was observed in a field of Lima beans grown on the government Horticultural Field Station farm near Beltsville, Maryland. From 5 to 10% of the plants showed unmistakable mosaic symptoms resembling in some ways the mosaic of garden beans. The plants were dwarfed and the yield noticeably reduced. Inoculation experiments made by rubbing the expressed juice from infected plants on the primary leaves of Lima beans yielded as high as 100% infection. Symptoms appeared in 6-7 days after inoculation. All attempts to infect the Stringless Green Refugee bean variety were unsuccessful, showing that it is evidently different from the common bean, *Phaseolus vulgaris*, mosaic. Attempts to transfer it to *Vicia faba* gave negative results, which show that it is probably different from the mosaic of several of the common legumes, as for example, red clover, white clover and white sweet clover. Inoculations to cucumbers and tobacco produced symptoms similar to cucumber mosaic. The identity of the Lima-bean mosaic has not been definitely determined, but some evidence indicates that it may be related to the cucumber mosaic.

Effect of Nutrients on Susceptibility of Tobacco Plants to Downy Mildew. R. G. HENDERSON.

In sand-culture experiments, tobacco plants grown in a solution low in nitrogen were very susceptible to downy mildew, as were also plants grown in a solution high in potassium. But plants grown in a solution low in potassium and relatively high in nitrogen were quite resistant. In these experiments the addition of an extra supply of a nitrate salt did not increase the susceptibility of the plants, as is usually presumed. It has been noticed in outdoor plant beds that downy mildew usually appears first on the stunted plants and on plants growing around old stumps in the bed where the plant food might be deficient.

Promising Fungicides for Tobacco Downy Mildew Control. R. G. HENDERSON.

Experiments in the greenhouse and in outdoor plant beds have indicated that cuprous oxide and benzoic acid, used with cottonseed oil emulsion, are quite effective for tobacco downy-mildew control. The cuprous oxide was used at the rate of 1 pound to 100 gallons of water and the benzoic acid at the rate of $\frac{1}{4}$ pound to 100 gallons of water. One % oil emulsion was used in both cases. Linseed oil and benzoic acid was used in one instance with good results. Pieric acid proved to be less effective than benzoic acid and was injurious to the foliage.

The Colored Zones Associated with Decay in Trees. HENRY HOPP.

Various polypores, subjected to controlled environmental influences in cultures, formed colored zones (black lines) whenever the mycelium was exposed concurrently to a large amount of air and to a considerable concentration of water, provided atmospheric oxygen was present. The zones consisted of brown, gnarled hyphal masses that arose from hyaline mycelium. Colored zones of *Fomes igniarius* in *Populus* and *Fagus* (12 decayed and 10 healthy trees were studied) did not enclose the longitudinal limit of decay, but occurred where the circumference of the decayed wood cylinder adjoined the surrounding band of slightly colored, noninfected wood. Here the mycelium was exposed to large concentrations of air and water, since the intracellular space of the healthy layer contained 26% to 80% water and that of the decayed wood only 7% water. The air within the decayed and healthy portions did not differ significantly in chemical composition. The band of noninfected wood, previously termed the invasion zone, was actually the peripheral layer of heartwood. Its presence, therefore, was not a criterion of fungous parasitism. The point of inoculation was deduced from the distribution of colored zones. Typical sporophores of *Fomes applanatus* formed when cultures were ventilated and exposed to 75% relative humidity.

The Problem of Drilling Dusted Seed: Effect of Graphite. JAMES G. HORSFALL and EARL L. ARNOLD.

Last spring internal force-feed grain drills sowing peas dusted with cuprous oxide were frequently choked, sometimes broken, as in the case of copper-carbonate-treated wheat. Seeding rate was reduced as much as 30 per cent, thus giving farmers the erroneous idea that the seed protectant was valueless or worse. Increased interfacial friction between the seeds, produced by separating their surfaces with a non-lubricating fungicidal dust, was responsible for the difficulties. The friction increased with dosage, with coverage as related to mixing time, with moisture content of the seed, and hence with the length of moist storage. Merely tumbling seeds in a power treater so scarifies the surface as to increase friction. Lubrication obviously is the solution. Water sprayed on is satisfactory provided it is not given time to penetrate the seed and raise the moisture content. Finely pulverized graphite (325 mesh) is better. It can be added during the treating operation, will not soak into the seed, and is so inert as not to interfere with germination or with the protective action of the chemical. Graphite has reduced the friction to normal for cuprous oxide-treated peas, spinach, and wheat, Semesan-treated cabbage, and copper carbonate-treated wheat.

Genetic Types of Resistance to Bacterial Wilt of Corn. S. S. IVANOFF and A. J. RIKER.

Bacterial-wilt-resistance studies (*Phytophthora stewartii*), based on large numbers of Golden Bantam inbreds and F₁ hybrids, various commercial crosses, open-pollinated sweet, dent, and flint corns, showed the existence of three types of resistance: vigor-correlated (vigor measured by height), lateness-correlated, and "true" resistance. The inheritance of resistance was expressed in general as follows: Hybrids produced from low-resistant inbreds showed lower degree of resistance than hybrids produced from high-resistant inbreds (the other two plant characters, vigor and lateness, for the two groups of hybrids being about the same). Hybrids produced from low-resistant inbreds showed greater resistance than either parent, the vigor of such hybrids apparently increasing their resistance. Hybrids produced from high-resistant inbreds showed a degree of resistance approximately equal to that of the more resistant parent. Hybrid resistance in general appeared to be influenced mainly by the resistant parent, the susceptible parent having little or no influence. Open-pollinated sweet-corn varieties showed approximately the

same degree of resistance as open-pollinated dent and flint varieties of the same height and lateness.

Are Tobacco Plants Affected with Mild Mosaic Susceptible to Other Strains of the Virus?

E. M. JOHNSON and W. D. VALLEAU.

Tests were made with Turkish tobacco plants to determine whether the presence of mild strains of tobacco mosaic would protect them against infection by more severe strains if the latter were introduced after the former had become established in the tip leaves. Plants having 6-7 leaves were inoculated with various natural strains of mild green tobacco mosaic and when infection was systemic, with the virus of white or yellow tobacco mosaic. Mild mosaic plants, inoculated on noninoculated leaves, with white or yellow mosaic developed white or yellow mosaic in the tip leaves in 10-19 days. If inoculations were made on mild mosaic-affected leaves, white or yellow mosaic developed in 20-60 days. Comparable plants inoculated with only white or yellow mosaic developed systemic infection in 7-10 days. Symptoms of white or yellow mosaic were only slightly less conspicuous in mild mosaic plants than in those having only white or yellow mosaic. The tests indicate that protection may be afforded individual cells or groups of cells because they probably are completely occupied by the first virus, but the plant as a whole does not develop immunity.

Virus Diseases of Peas. FOLKE JOHNSON and L. K. JONES.

Common mosaic and severe mosaic of peas belonging to the "sprekel" and "marmor" types, as described by Merkel, occur in Washington. Based upon symptomology as well as longevity, host range, and percentage transmission tests these are caused by distinct viruses. Cross inoculations have been made with the viruses from pea, alfalfa, white clover, red clover, sweet clover, alsike clover, beans, vetch, horse bean, chick pea, and lentils. It was found that all these hosts except chick pea were susceptible to infection by the severe mosaic virus, while alfalfa, alsike clover, beans, and horse bean were susceptible to infection by the common mosaic virus. Tests with 12,000 seedlings grown from seed collected from virus-infected plants showed that these viruses are rarely transmitted through the seed. Preliminary field tests with 488 varieties and strains of peas showed no varieties immune, but 48 exhibited some degree of resistance.

Relation of Leaf Rust (Puccinia triticina) Infection to the Rate of Transpiration in Two Varieties of Wheat. C. O. JOHNSTON and E. C. MILLER.

Greenhouse experiments conducted at Manhattan, Kans., proved that heavy leaf-rust infection of a susceptible wheat (Pusa No. 4, C. I. 8899) initiated during anthesis increased transpiration an average of 17% for periods of 24-48 hours. Transpiration in a resistant variety (Pusa 52 x Federation, C. I. 11764) was increased an average of 3.1%. Infection of this variety was expressed principally by flecking and necrosis, accompanied by a few minute uredia. Water-loss measurements, made from 8 a.m. to 6 p.m. and from 6 p.m. to 8 a.m., showed that heavily rusted plants of the susceptible variety transpired an average of about 78% more water at night than did rust-free control plants. Loss from infected plants during the day was slightly lower than from non-rusted controls. This apparently was due to artificially induced closing of many stomata of infected plants caused by rust appressoria. The results indicate that the greater water loss from rusted susceptible plants is traceable to increased cuticular transpiration, induced by rupturing of host tissues. During the day the relatively small cuticular transpiration was masked by the high stomatal transpiration; at night it was easily observable. In the resistant variety there was no consistent quantitative difference in transpiration by infected and

noninfected plants during day and night. (Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station.)

The Relation of Zinc Sulphate to Injury from Peach and Apple Sprays in the 1935 Season.—K. J. KADOW AND H. W. ANDERSON.

Comparisons of ZnSO_4 with other materials were made on Ben Davis apples. ZnSO_4 caused severe russet in about the same degree as CuSO_4 . Neither lime nor flotation sulphur caused russet. One thousand fruits were examined in each plot except the check, which was so severely infected that only 412 apples were available at harvest. In the control of scab, lime was 20% effective, ZnSO_4 66%, CuSO_4 94.5%, and flotation sulphur 91.8%. ZnSO_4 did not materially influence codling moth control or total residue at harvest. Lead arsenate and lime, with varying amounts of ZnSO_4 , were applied to peach trees to determine the amount of ZnSO_4 necessary to correct arsenical injury. Injury in all plots was much more severe than usual, due to the extremely damp season. The results showed little difference between the various ZnSO_4 plots. Injury was severe in the lead arsenate-lime plot. The check was in excellent condition. In laboratory chemical studies, practically complete elimination of water-soluble arsenic from the standard lead arsenate-lime spray mixture was obtained by additions of as little as $\frac{1}{2}$ lb. ZnSO_4 to 100 gal. of water.

Progress of Studies of the Epidemiology and Control of Cherry Leaf Spot.—G. W. KEITT, E. C. BLODGETT AND R. O. MAGIE.

Results of studies of (1) relations of time and temperature in the inoculation chamber to incidence of infection and (2) length of incubation periods at controlled temperatures are presented graphically. Comparative trials of Bordeaux mixture and lime sulphur for four years on replicated Montmorency plots indicate the importance of cumulative effects. Bordeaux was much the more efficient spray for disease control, due largely to longer duration of effectiveness per application. Little difference in size of fruit was found between plots sprayed continuously with Bordeaux and lime sulphur, respectively. In certain cases where Bordeaux and lime sulphur were alternated annually, the lime-sulphur-sprayed fruit was substantially larger than that of corresponding Bordeaux plots. Nonsprayed or lime sulphur plots that had severe leaf-spot attacks shortly before harvest bore the largest fruit. Total sugar content of such fruit tended to be less than that of the smaller fruit from Bordeaux plots, where leaf spot was controlled. However, under some conditions, Bordeaux spraying appears to lead to actual reduction in the size of fruit. In these experiments on Montmorency any advantage of lime sulphur regarding fruit size appears to have been more than offset by greater fruitfulness following suitable use of Bordeaux.

*Cytological Studies of the Parasitism of Two Monoconidial Isolates of *Venturia inaequalis* on the Leaves of Susceptible and Resistant Apple Varieties.*—G. W. KEITT AND C. J. NUSBAUM.

Leaves of suitable varieties were infected in the greenhouse and studied cytologically. In all cases a minute infection hypha penetrated directly through the cuticle, and a dendritic subcuticular mycelium developed. Later developments varied with isolate and apple variety. Isolate 22a parasitized Yellow Transparent vigorously, Fameuse moderately: 17a parasitized Fameuse vigorously, Yellow Transparent moderately. Missouri Pippin was resistant to both isolates, reacting differently to each. In very susceptible

leaves, host cells showed little abnormality until about 10 days after infection. Then progressive depletion of plastids and cytoplasm, attended by increased vacuolation, began to appear in the upper palisade layer at the middle of the lesion. This impoverishment gradually spread throughout the area underlying the fungus, and was followed by necrosis. The fungus showed no apparent injury until the host cells had died. In moderately susceptible leaves the fungus developed less vigorously, impoverishment of the host cells was less rapid and severe, and little necrosis occurred. In resistant leaves (Missouri Pippin), fungus growth was sharply restricted. Localized epidermal necrosis occurred with isolate 22a; none with 17a. The parasitism of *Venturia inaequalis* is especially noteworthy in that these delicately balanced host-parasite relations are maintained without the fungus penetrating beyond the subcuticular position.

A Cowpea Resistant to Fusarium Wilt and Nematode Root Knot.—JAMES B. KENDRICK.

Fusarium wilt, caused by *Fusarium bulbigenum* v. *tracheiphilum* and root knot, caused by *Heterodera marioni*, are limiting factors in the production of blackeye cowpeas in California. Field trials in 1928 and 1929 showed the Virginia blackeye cowpea, *Vigna sinensis*, highly resistant to Fusarium wilt, but not adapted to California cultural practices. Hybrids from crosses between the California blackeye and the Virginia blackeye were highly resistant, but tended towards extreme vegetative vigor, irregular and light seed production, and a somewhat small pea. Repeated planting on sick soil, extensive single plant selections, and back-crossing to the susceptible California blackeye have resulted in several strains highly resistant to both Fusarium wilt and root knot with increased production, less vigorous vines, and commercially desirable seed types. Since these strains resulted from crosses between the California and Virginia blackeye varieties, the resistant types have been designated the "Calva" blackeye, given appropriate numbers, and are being distributed to representative growers.

A Vascular Fusarium Disease of Radish.—JAMES B. KENDRICK AND WILLIAM C. SNYDER.

In April, 1934, a disease resembling cabbage yellows (*Fusarium conglutinans*) was observed in a seed field of white Chinese winter radishes in San Benito County, California. Diseased plants showed yellowing and dropping of the leaves, which was frequently confined to one side of the plant, vascular discoloration, severe stunting, and often death. A *Fusarium* was readily isolated from the discolored vascular tissue. White Chinese winter and white icicle radishes grown in steam-sterilized soil, inoculated with the *Fusarium* showed over 90% infection, while early Jersey Wakefield cabbage and Jersey kale grown in the same soil showed no evidence of the disease. Long black Spanish, California mammoth white, long scarlet, China rose winter, and French breakfast varieties of radishes also showed a high degree of susceptibility under greenhouse conditions. Macroscopic and microscopic examinations of tested single-spore cultures of the *Fusarium* show it to be a member of section *Elegans*. Terminal and intercalary chlamydospores and microconidia are produced abundantly, and the macroconidia are of the *Elegans* type. The production of deep purplish pigmentation and occasionally dark sclerotia definitely differentiate it from the *F. conglutinans* type, according to Wollenweber's *Fusarium-Monographie*.

Entry of Fusarium moniliforme and Cephalosporium acremonium into Growing Corn Ears.

—BENJAMIN KOEHLER.

Ears of dent corn well covered by the husks and free from visible signs of rot were dissected in five stages of development, and parts plated. In the 500 ears plated,

the possibility of entry through the shank was ruled out except in a very few questionable cases. *Fusarium moniliforme* entered all infected ears through the tip end, passed downward along the silks, then inward along the surface of the kernels, then along the bracts and pedicels, and finally entered the vascular cylinder of the cob. It had been entered in 8 per cent of the ears in the milk stage and 46 per cent of the mature ears. When ears had been attacked by earworms only at the tip, and at the same time were free from obvious mold, infection was increased to 48 and 90 per cent, respectively. *Cephalosporium acremonium* was found in the well-protected, worm-free ears almost as frequently as *F. moniliforme*, but it was not greatly augmented by injury at the ear tips. Its path of entry also was the same in at least 90 per cent of the infected ears.

Anthracnose-resistant Watermelons.—DUKE V. LAYTON.

Inoculations with *Colletotrichum lagenarium* were made on edible varieties of *Citrullus vulgaris* including the available domestic varieties and an extensive collection of foreign varieties. In 1932, anthracnose-resistant strains were obtained from seed of watermelon native to Umtali, South Rhodesia, Africa. With crosses between this strain and susceptible varieties, resistance to anthracnose was found to be dominant in the F_1 , and segregated in the ratio of 3 resistant to 1 susceptible in the F_2 generation. When the F_1 progeny was back-crossed on the susceptible parent, segregation for resistance and susceptibility was 1:1. Similar results were secured with reciprocal crosses. Since the anthracnose-resistant strains are susceptible to *Fusarium niveum*, crosses have been made on wilt-resistant varieties in order to secure resistance to both diseases. Watermelon selections have been isolated from these crosses that are homozygous for resistance to both anthracnose and wilt.

The Relation of Soil Temperature to the Development of Fusarium Wilt of Muskmelon and the Demonstration of Internal Seed Transmission.—J. G. LEACH.

Unlike most *Fusarium* wilts, muskmelon wilt does not depend upon high soil temperatures for best development. The optimum temperature for the development of the pathogen on agar lies between 27 and 30° C. Muskmelon seeds did not germinate well in soil below 20° C., and seedlings grew best at 35° C., the highest temperature tried. Within this range the wilt was most severe at the lower soil temperatures. When a plant is killed by wilt late in the season the pathogen often invades and rots almost mature muskmelons. Seed taken from decayed fruits and surface-disinfected yielded pure cultures of the pathogen when germinated on agar. Plants grown from such seed in steamed soil were wilted in the seedling stage. The fungus enters the seed at the point of attachment to the placenta and develops in the parenchyma of the spermoderm, but does not penetrate the perisperm nor infect the embryo. If the disease occurs in the seed-producing regions, seed infection provides means for long-range dissemination.

Microorganisms Antibiotic or Pathogenic to Cereal Rusts.—M. N. LEVINE, R. H. BAMBERG, AND R. E. ATKINSON.

A bacterial parasite, first found associated with urediospores of *Puccinia graminis* isolated from garden slugs, has since been obtained from many field rust specimens. Fairly common throughout the Mississippi Valley, it also occurs eastward to Virginia and westward to Oregon. In the field it attacks stem rust more frequently than leaf rust. In the greenhouse it attacks all cereal rusts. Pycnia, aecia, and uredia may be totally destroyed. The organism, of the genus *Bacillus*, inhibits development of rust on both adult plants and seedlings. High relative humidity favors its rapid development. An undetermined imperfect fungus, also first found associated with slugs, envelops

rust pustules with thick, floccose hyphal wefts, at first pinkish gray, later turning black. In the greenhouse, under high relative humidity, it soon obliterates pustules but is rarely found in nature. Recently a species of *Trichoderma* has been found that overgrows rust pustules under bell jars in the greenhouse. Its hyphae penetrate the urediopores through the germ pores. In hanging drops urediospore germination is completely inhibited, but on host plants rust infection is not prevented, though its further development is hindered. Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Division of Plant Pathology and Botany, University of Minnesota.

Gall Production in High and Low Carbohydrate Tomato Plants.—GEORGE K. K. LINK AND HAZEL W. WILCOX.¹

Tomatoes grown in quartz sand received -N and +N nutrient solutions. When the former (hard) plants showed abundant starch and no nitrate, and the latter (soft) plants abundant nitrate and no starch, 10-, 13-, and 15-week-old plants were inoculated at the top, middle, and base with uniform doses of *Phytomonas tumefaciens*. After 5 weeks, cross-section measurements were made and used to determine percentage of gall area relative to normal stem area. It was found that: 1) This percentage was larger in -N than in +N plants at each level inoculated; 2) In both -N and +N plants this percentage was greatest in the 10-, less in the 13-, and least in the 15-week-old plants; 3) In -N plants the top gall percentage was larger than the basal-gall percentage. In +N plants this difference applied to the 13- and 15-, but not to the 10-week-old plants. These showed equal percentages. The results suggest that gall production in tomato plants is favored by substances relatively abundant in -N (hard) plants and comparatively lacking in +N (soft) plants. In the presence of these substances gall proliferation is apparently proportional to the growth rate of the axis level inoculated.

Comparative Physiology of Pathogenic and Nonpathogenic Crown-Gall Bacteria.—MARY M. LYNEIS, A. J. RIKER, AND S. B. LOCKE.

Studies have been made on the comparative physiology of 2 strains of *Phytomonas tumefaciens*, which are respectively pathogenic and nonpathogenic to tomato. The strain that does not infect tomato was found to induce galls on a tobacco hybrid. Likewise, since the temperature range 28-30° C. is critical for crown-gall development on tomato, studies were made of both cultures, respectively, above and below this range. The culture that is pathogenic at 27° but not at 32° C. on tomato, induces galls at both temperatures on the tobacco hybrid. Bacteriological characters examined have included: (1) utilization of certain sources of nitrogen and of carbon, respectively, (2) production of CO₂ and of H₂S, (3) agglutination, and (4) changes in osmotic pressures in yeast-extract and host-plant-extract media. Various working hypotheses proposed for the cause of cell stimulation by *P. tumefaciens*, based on some of these characters, have not received confirmation in these studies. The culture nonpathogenic to tomato appears to grow in the tomato plant during at least the first 2 weeks approximately as well as the pathogenic culture, indicating in the early stages no bacteriostatic effect by the plant on this culture.

Relative Adherence of Cuprous Oxide and Other Copper Fungicides.—R. O. MAGIE AND JAMES G. HORSFALL.

Tests have been made during the past 2 seasons, comparing by means of curves the initial adhesiveness and resistance to weathering or adherence of several copper fungicides,

¹ Supported in part by a grant from the Rockefeller Foundation to the University of Chicago.

also the effect of the addition of emulsified cotton-seed oil. The sprays that contained equivalent Cu contents were tested on apple and cherry foliage in the orchard. Two hundred leaves per tree on each of 5 or 6 sampling dates were analyzed for copper by the Elvehjem and Lindow modification of Biazzo's method. The fungicides stood in the descending order of their ability to adhere to the leaf approximately as follows: Cu_2O plus oil, Bordeaux mixture plus oil, Bordeaux mixture, Cu_2O , copper oxychloride (KK), copper phosphate, copper-ammonia-silicate plus oil, and copper-ammonia-silicate. The addition of emulsified cotton-seed oil to Cu_2O or to the copper-ammonia-silicate spray nearly doubled the initial adhesiveness. The term "initial adhesiveness" is here applied to the ability of the spray to become attached to the leaf in contrast to "adherence," which is applied to the resistance of spray residues to weathering.

Perennial Phlox Resistant to Powdery Mildew.—E. B. MAINS.

In 1933 powdery mildew severely attacked perennial phlox in Ann Arbor. In one garden a plant, apparently volunteer, was noticeable on account of its freedom from mildew. In 1934 and 1935, this plant and seedlings apparently arising from it were studied in contrast with named varieties. The varieties Africa, Commander, E. Pritchard, Graf Zeppelin, Lilian, Marchal French, Miss Lingard, Mrs. W. Van Beuningen, Nicholas Flammel, Paladin, Rynastroom, R. P. Struthers, Thor, Von Lassburg, and Widar were very susceptible. Enchantress, Emair Maeks, Leo Schlageter, Pastel, Saladin, and Milly Van Hoboken were somewhat less susceptible. Columbia mildewed slowly, finally becoming moderately infected. No mildew was evident on the resistant plant throughout the study. Of the seedlings, 4 were very susceptible, 4 moderately susceptible, 7 showed various degrees of resistance. Nine were highly resistant showing no evidence of mildew.

Peach Yellows and Little Peach Studies.—T. F. MANNS.

Of the several insects tested, the plum hopper, *Macropsis trimaculata*, is the only one found disseminating the viruses of yellows and little peach. A survey of many Eastern and Central States (Ill., Ind., Ohio, Penna., Del., Md., and N. J.) shows plums are the host plants, though it is found on many neglected peach and apricot trees. We have found the insect most common on the Oriental plum, *Prunus salicina*; in some cases as many as 5,000 to 20,000 hoppers per tree. We have shown that plum may carry either or both of the viruses of yellows and little peach. Some of the Oriental plum varieties may carry these viruses and live for many years and at the same time breed many hoppers and disseminate these diseases. When both viruses are budded into peach or plum at the same time, yellows symptoms are the first to appear but later the yellows is suppressed and little-peach symptoms predominate. When plum hoppers, *Macropsis trimaculata*, that had fed on trees carrying yellows or little peach, were crushed and injected by hypodermic needles into growing twigs or leaves of peach no infection resulted. The incidence of infection, was very low whether trees were exposed to many or few hoppers (100, 25, 10, or 5) carrying the viruses of either yellows or little peach; after repeating these trials 3 successive years, the percentage of infection reached only 10–15% on peach seedlings. With the Elberta variety it was 25 to 50 per cent.

The Action of Fungous Spores on Bordeaux Mixture.—S. E. A. MCCALLAN AND FRANK WILCOXON.

The conclusion that fungous excrete substance capable of rendering the copper in Bordeaux mixture soluble, has been confirmed by the use of sensitive chemical tests capable of detecting and estimating concentrations of copper of the order of 1 part in 10 millions. It was found that distilled water would dissolve copper from films of dried

1:1 Bordeaux giving solutions containing 0.2 p.p.m. of copper. If however the Bordeaux films were treated with water in which spores (*Sclerotinia fructicola*, *Botrytis paeoniae*, *Neurospora sitophila*) had remained overnight, with subsequent filtration, 2-5 p.p.m. of copper were found in solution, an amount sufficient to prevent germination. In obtaining the spores a vacuum technique was employed that avoided contamination with the medium on which the fungus was cultured. A sufficient amount of spore excretions was collected for chemical examination. From the lead precipitate malic acid was identified as the hydrazid by its melting point. A mixed melting point with an authentic sample showed no depression. Malic acid, even in neutral solution, was able to bring large amounts of copper into solution from dried Bordeaux. Other hydroxy acids behave similarly. It is concluded that malic, and perhaps other, acids contained in spore excretions lead to the solution of sufficient copper from Bordeaux mixture to prevent spore germination.

Collecting Microorganisms from Winds Above the Caribbean Sea. FRED C. MEIER.

Pure cultures of various species of fungi, and spores embedded in petrolatum on glass slides, were obtained by the author in July from airplanes at 87 positions over Central American waters and land areas. This study, made possible by informal cooperation between Pan American Airways, the National Geographic Society, and the United States Department of Agriculture, calls attention to the trade winds as possible agencies in the dissemination of microorganisms. Sterile plates, occasionally obtained from exposures during and after rain squalls, at altitudes between 200 and 800 feet while only a few miles to leeward of the outer islands of the Caribbean, demonstrate the effectiveness of frequent showers in reducing spread of organisms by surface winds. However, viable spores taken from air currents above the clouds show that dissemination of certain fungi may occur regardless of and ultimately aided by rainfall at lower levels. This is confirmed by cultures obtained at points along the 500 mile course over water between Jamaica and Colombia and at a point some 50 miles east of Panama. Depending on exact wind direction, material collected on these last 2 courses may have traveled at least 500 to 700 miles across open water. (Cooperative Investigations, Bureau of Plant Industry and Weather Bureau, U. S. Dept. of Agriculture, Pan American Airways, and National Geographic Society assisting.)

Control of Brown Patch in Turf by Fanning. JOHN MONTEITH, JR. and MARY E. REID.

Brown-patch of turf caused by *Rhizoctonia solani* is most prevalent and destructive during periods of excessive heat combined with high humidity and heavy rainfall. For several years it has been observed that adequate circulation of air reduces the damage from certain turf ailments, especially brown patch. Openings cut through trees and underbrush in the direction of prevailing winds have repeatedly resulted in greatly reducing brown-patch damage. Tests have been made with fans to provide air circulation over bent turf during the night. In all cases a decided checking of the disease was noted resulting in an increase in growth from 10% to 40%. The benefit from fanning is probably chiefly that of drying the grass, thereby limiting mycelial growth. Fanning reduced soil temperature from 3° to 5° F. It did not materially affect the amount of grass produced where disease was not a factor. During periods of excessive rainfall it was necessary to supplement fanning with mercury fungicides to accomplish complete control. Propeller-type fans, delivering a current of air from 2 to 12 miles per hour appear to offer practical aid in controlling brown patch on many areas of turf located in air pockets. The work further emphasized the importance of making provisions for natural air circulation over turf.

A Partial-Vacuum Method for the Inoculation of Wheat and Barley with Loose Smuts.
M. B. MOORE.

An apparatus has been devised by means of which individual heads of wheat or barley are subjected to partial vacuum while completely submerged in an aqueous suspension of chlamydospores of the loose smuts and by means of which as many as 30 heads can be inoculated in one hour. A glass inoculating chamber is connected by its upper end to a hand-operated vacuum pump and fitted at its lower end with a No. 7 split-rubber stopper by means of which the wheat or barley head may be sealed in the chamber. An inoculum-supply flask is placed lower than the chamber and connected with it through a rubber tube and pinch cock. When the pinch cock is opened, suction from the pump draws inoculum into the chamber. When it is closed, operation of the pump results in alternating partial vacuum and normal pressure, which replaces the air in the florets with the spore suspension. Susceptible varieties of wheat and barley averaged 58 and 43% smut, respectively, when inoculated by this method.

Pathogenicity of Different Collections of Ustilago tritici and U. nuda. M. B. MOORE.

Spore material of *Ustilago tritici* was collected from Minnesota, North Dakota, Texas, and Mexico. Each of 12 collections was inoculated by the partial-vacuum method into 6 to 8 heads of each of 9 differential varieties of wheat. Results indicate that the 12 collections comprise 5 parasitic races. In general, individual collections inoculated into particular varieties caused either no infection or from 20 to 100% infection, making possible a very sharp and definite separation of physiologic races. The results of similar inoculations on barley were not consistent enough to permit the separation of parasitic races, but did indicate that Trebi is rather highly resistant to the 9 collections of *Ustilago nuda* used.

The Influence of Cercospora-infested Soil in Relation to the Epidemiology of Cercospora Leaf Spot on Sugar Beets. C. M. NAGEL.

Studies have been made on the growth, longevity, and pathogenicity of *Cercospora beticola* in sterile soil cultures, and with naturally infested soil obtained from the field and used in greenhouse experiments. *C. beticola* was grown on 5 types of soil: peat, basic black loam, acid black loam, neutral black loam, and black loam low in organic matter. The best mycelial growth was obtained on the peat soil. The pH value of the cultures varied, although not widely, and were maintained at a temperature of 24-26° C. The cultures were kept for 5 weeks in a closed moist chamber, to eliminate drying of the soil; then they were placed at ordinary laboratory temperatures for the remainder of the experiment. The cultures soon attained an air-dry condition and mycelial growth was checked. At the end of 27 months nutrient-agar transfers showed that the organism was still viable. When sugar-beet seedlings were directly inoculated with the soil cultures, typical disease symptoms developed. A technique was developed for isolating the pathogen directly from the soil by means of the host plant. In greenhouse experiments, when sugar beets were grown on naturally infested soil, typical symptoms developed on both cotyledonary and true leaves.

Progress of Studies of Eradicant Fungicides in Relation to Apple Scab Control.—D. H. PALMITER and G. W. KETT.

Fall treatments of apple leaves with copper-lime-arsenite sprays were somewhat less effective than they were last season in preventing development of perithecia of *Venturia inaequalis*, due chiefly to lessened dosage and exceptionally favorable climatic conditions for perithecial development. The more effective formulae reduced the number of peri-

thecia by 95-98.4%. No serious spray injury occurred. The effect of limited ascospore inoculum in relation to epidemiology was studied on several hundred young Wealthy and Northwestern Greening trees. Plot 1 received a copper-lime-arsenite fall spray in 1934 and was nearly free of perithecia in 1935: plot 2, about 100 yards away, received no fall spray and had a light ascospore inoculum. Neither plot was sprayed in 1935. On the basis of counts, and taking plot 2 results as 100%, the following percentage reductions in amounts of scab were indicated for plot 1: May 31 (petal-fall), Wealthy leaves 96: June 24, Wealthy fruit 91: June 29, Wealthy leaves 89, Northwestern Greening leaves 96. On Sept. 4, 89% of Wealthy fruit of plot 1 was scab-free: plot 2 bore insufficient fruit for counts. Climatic conditions for scab development in 1935 were good.

Distribution and Prevalence of Ozonium Root Rot in Shelter-belt Zone of Texas.—GEORGE L. PELTIER.

In view of the insidious nature of the indigenous fungus *Ozonium omnivorum* the infested areas within the Shelter-belt zone extending into southern Oklahoma and Texas in advance of planting were mapped with the object of avoiding these areas or employing resistant trees. The susceptible plants employed as indicators of infested lands consisted of the more common endemic herbaceous plants, shrubs, and trees, together with annual and perennial weeds and many crop plants. The universal distribution of three susceptible weeds (nightshade, ragweeds, and lambsquarters) in virgin, pasture, waste, and cultivated lands, along railroad embankments, roadsides, fence rows, ditches, washes, banks of dry creeks and on flood plains of larger streams, as well as the presence of conidial mats, aided very materially in detecting *Ozonium*. A section of land was deemed infested when one or more diseased plants revealed the characteristic mycelial weft on the roots, or when conidial mats were found. On the basis of the above procedure the approximate limits of root-rot infestation were found to be south of the 34th degree of latitude and east of the 100th meridian. In the main the prevalence of root rot was much more pronounced in the better agricultural lands and in the valleys. The sharpness with which the infested and noninfested areas were delimited was especially striking. (Special investigation, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture).

Progress of Spraying Experiments for Control of Apple Fire Blight.—J. A. PINCKARD, G. W. KEITT, and A. J. RIKER.

Potentialities of spraying as a control measure for fire blight have been studied at Gays Mills, Wis., for three seasons. Applications were made throughout the period from the "green-tip" stage of bud development until early August, including sprays in bloom. Lime sulphur as used against scab was of no value against blight. Suitable programs of Bordeaux, 1: 3: 50, and copper phosphate-bentonite-lime mixture, 2: 2: 4-50, appeared in most cases to reduce the amount of blight substantially (commonly by about $\frac{1}{2}$ or $\frac{3}{4}$), but did not completely control it. Blossom and post-blossom sprays were important. The effectiveness of spraying varied much with seasonal and other conditions. The programs used were ineffective under the most severe conditions encountered. On Fameuse in 1935 Bordeaux, 1: 3: 50, occasioned severe russet and the copper phosphate mixture did not control scab satisfactorily. The results thus far obtained do not warrant recommendations for practice. However, they suggest substantial possibilities for spraying as a supplement to other means of blight control. For regions requiring frequent scab sprays during the critical period for blight infection, the desirability of a material that is effective against both scab and blight is indicated.

Crown Girdle of Pear Trees.—A. G. PLAKIDAS.

For several years, a disease has been affecting the Chinese sand pears in Louisiana. It is manifest as a bark canker at or near the ground line. The canker spreads irregularly, but more rapidly up and down the trunk, sometimes extending to the crotch and downward to the main roots, eventually girdling and killing the tree. In later stages of the disease, a white basidiomycetous fungus usually appears on the affected part of trunk and crown, forming white plaques between wood and bark. Inoculation tests have shown that this fungus is a secondary invader, as it fails to produce infection in living bark. Another fungus, probably *Dothiorella ribis*, is found constantly associated with the disease. Repeated inoculations with this fungus into branches and trunks of pear trees have resulted in large cankers, like those found on naturally infected trees. Observational evidence indicates a connection between freezing injury and the disease, the fungal infection starting at points of injury and spreading to sound bark. The disease has been noted also on apple and tung-oil trees. Good control has been obtained by scraping the affected bark and painting the wound with Bordeaux paste.

Effects of Some Copper Compounds on the Control of Bacterium pruni and on the Peach Tree.—R. F. POOLE.

On the basis of tests conducted during the past 3 years copper phosphate, ammonium copper silicate (Coposil), and red copper oxide (Cuprocide) have given more satisfactory reduction of *Bacterium pruni* infection than any of the many chemicals previously tested. Infection on sprayed trees was reduced more satisfactorily on fruit than on foliage. Fused bentonite sulphur, potash fish oil soap, and emulsified oils have improved the adhesive qualities of these chemicals without inducing injury or reducing the control values. Injury to foliage caused by all copper compounds used was prominent following applications before May 15. It also developed slightly late in the autumn. There was no injury throughout the summer months regardless of the strength and amounts applied.

A Tuber Rot of Southern Potatoes.—G. B. RAMSEY.

An unusual type of tuber rot was noted in South Carolina potatoes on the Chicago market in June, 1931. Yellowish brown, sunken, definitely circular lesions varying from $\frac{1}{8}$ to $\frac{1}{2}$ inch in diameter were characteristic. These spongy pocket-like lesions penetrated to a depth of $\frac{1}{8}$ to $\frac{1}{2}$ inch. The larger lesions showed concentric zonation of light and dark brown tissue on the surface, as well as in the interior. A scanty, fine, white mycelium was apparent on the surface of the larger lesions. From the interior of spongy pockets of all sizes pure cultures of an unidentified fungus have been obtained. This organism has produced neither sclerotia nor spores of any kind. The pathogenicity of the fungus has been proved by inoculation experiments.

Effects of Seed Treatment on Disease-free and Diseased Seed Corn.—C. S. REDDY.

Experiments in the greenhouse and field indicate that seed treatment may increase the yields from disease-free seed by inhibiting the action of pythiaceous fungi in the soil. Adverse conditions for germinations, such as low temperatures, were necessary in order to obtain beneficial results from seed treatment. In 1935, April 20 and 27 field plantings of disease-free seed corn were benefited 13.4 and 23.4 bushels per acre. Seed-treatment experiments in relation to rate of planting, including nearly disease-free, *Diplodia*-infected, and at times *Basisporium*-infected, and *Gibberella*-infected seed corn, were conducted during the period 1930 to 1934 at Ames, Iowa. Benefits from seed treatment were greater in comparisons with field stands less than the most productive ones,

being 4.4, 3.3, 2.7, and 1.1 bushels per acre, than with stands greater than the most productive ones, 2.5, -1.3, 1.9, and -0.7 bushels per acre. These latter differences show two mathematically significant increases and no significant decreases, which indicates that the plants from treated diseased seed were more productive than those from nontreated diseased seed. The increases could not have come from increasing the stands because the comparisons are within stands where an increase should produce a decreased yield.

Flax Seed Treatment.—C. S. REDDY.

Investigations of the causes of flax failures because of poor field stands have indicated that the seed-rotting and seedling blight organisms are not principally seed-borne but soil-borne pythiaceous fungi. Seed treatment has proved efficacious in this case because flax becomes resistant to these attacks very soon after germination. The time of delimitation of susceptibility is more sharply marked when germination occurs in cold soil. This, in part, accounts for recommendations of early sowing and for favorable results from seed treatment only when the sowings are early. In 1935, seed of nine varieties of flax, treated with New Improved Ceresan at the rate of one-half ounce per bushel as compared with the same not treated, produced 112% more plants on heavily infested corn land and 30% more on lightly infested corn land. In two experiments on fallow, treated seed, as above, produced 74 and 132% more plants than nontreated seed. April sowings of flaxseed, involving 4 varieties and 889 replications, gave yield increases of 17 and 24%, respectively, following seed treatments with Ceresan and New Improved Ceresan, the latter costing one-fourth as much as the former.

*Electrical Potentials Found in Studies on *Phytomonas tumefaciens* and Related Organisms and on Crown Gall.*—A. J. RIKER, J. A. PINCKARD, and I. L. BALDWIN.

Investigations have been continued on the oxidation-reduction potentials induced in liquid media by certain bacteria. The single-cell cultures used included 2 cultures of *Phytomonas tumefaciens* that were pathogenic and nonpathogenic on tomato, respectively; 1 of *P. rhizogenes* and 1 of *Bacillus radiobacter*. Each was used in 3 trials with yeast-extract, 4 with tomato-stem-extract, and 4 with carrot-extract media. The readings were made with a vacuum-tube potentiometer at frequent intervals up to 14 or 28 days, depending on experimental requirements. Each of the cultures reduced the oxidation-reduction potential, respectively, in each of the media employed suggesting that such bacteria growing within injured tissue may have a reducing effect. However, no correlation appears between potentials and pathogenicity. From the standpoint of the plant, over 50 measurements made, with a platinum electrode inserted into the crown-gall tissue, showed without exception a higher potential than that on a similar electrode in contiguous stem tissue. These results indicate a greater capacity for energy exchange in crown-gall tissue than in neighboring noninfected tissue.

*The Abscission of Pear and Apple Blossoms in Relation to Infection by *Erwinia amylovora* and *Phytomonas syringae*.*—H. R. ROSEN.

To the extreme rainfall and cloudy days of April and May, 1935, has been attributed the relatively poor setting of fruit in the Ozarks this season. However, a careful examination of abscised flowers gathered while still loosely clinging to the clusters or from the ground beneath the trees, showed in many instances more or less localized blackish spots on various parts of the receptacles. Out of 64 isolations from abscised Kieffer and Garber blossoms gathered in two counties, 46 yielded pathogenic bacteria

as revealed in artificial inoculations with pure cultures. Of these 46, 29 were typical *E. amylovora* and 17 were *P. syringae*. In 5 isolations both types of bacteria were obtained. Likewise, counts made of several hundred abscissed Kieffer blossoms showed localized blackish spots in approximately 85 per cent of the blossoms. It appears that the hindrance of pollination or of setting of fruit during wet weather is not wholly a matter of adverse weather conditions.

Acquired Resistance of Potato to Latent Mosaic.—E. S. SCHULTZ AND W. P. RALEIGH.

Healthy Katahdin tuber and shoot, grafted onto latent mosaic Green Mountain, manifest necrosis of foliage and tubers (certain other potato varieties and seedlings give the same reaction to latent mosaic). Tuber progeny of the necrotic shoots develop plants manifesting different stages of necrotic symptoms; some of the shoots may be severely necrotic resulting in premature death, other shoots may show a few necrotic spots and more or less irregular light green areas. Mottled Katahdin shoots grafted onto latent mosaic Green Mountain develop mottling and mild necrosis. Likewise healthy Katahdin shoots, grafted onto mottled Katahdin, manifest mottling and a few necrotic spots. Inasmuch as the mottled and mildly necrotic Katahdin fail to develop severe necrosis on subsequent grafts on latent mosaic Green Mountain, it appears that mottled Katahdin has acquired resistance to latent mosaic. Furthermore, these reactions indicate that the latent mosaic virus harbored by the mottled Katahdin may either be attenuated or represent a weak type of the latent-mosaic virus group.

Reaction of a Green Mountain Potato Seedling to Composite Infections of Mild and Crinkle Mosaic and Different Types of Latent Mosaic Virus.—E. S. SCHULTZ AND W. P. RALEIGH.

By using different types of latent-mosaic, which can be classified according to severity of reaction on *Datura stramonium* into faint, medium, severe and virulent types, it is possible to produce different types of symptoms on Green Mountain seedlings with inoculations involving these types of latent virus and mild or crinkle-mosaic viruses. Mild-mosaic virus plus severe latent-mosaic virus causes a distinct mottling and ruffling on a Green Mountain seedling, while mild-mosaic virus plus faint latent-mosaic virus causes mottling but no ruffling. In the absence of latent-mosaic virus the mild-mosaic virus causes no mottling but slightly light green and rugose foliage on a Green Mountain seedling. These reactions indicate that mild mosaic and latent mosaic are caused by different groups of viruses and that different symptom expressions can be caused by combinations of viruses of different mosaic groups.

Infection Studies With Sclerotinia fructicola.—M. A. SMITH.

From histological studies it has been shown that infection by *Sclerotinia fructicola* of the non-brushed surfaces of the peach varieties Elberta, Hale, and Early Crawford occurs for the most part by way of hair sockets. Stomatal infection was not commonly observed. Direct infection through the cuticle of normal epidermal cells was only rarely seen. The minimum time for infection of peach fruits from which excess hair had been removed by brushing was found to be 4½ hours. Stomatal and direct infections were not common. The most common method of entry of germ tubes was by way of the hair sockets. The greater rapidity with which infection occurred on brushed fruits appeared to be correlated with the relative accessibility of the brushed-fruit surface to germinating conidia of *S. fructicola*.

Studies on the Life Cycle and Control of Fabraea maculata on Kieffer and Garber Pears.
—M. A. SMITH AND M. C. GOLDSWORTHY.

Studies during the past 2 years in Missouri and Maryland indicate that the fungus, *Fabraea maculata*, found in these localities, depends principally on the overwintering of the conidial stage in bark cankers for a continuance of the life cycle rather than upon the production of ascospores in the fallen leaves. These conclusions were reached after detailed studies were made of the initial infections and of the prevalence of the ascigerous stage. Nondiseased trees did not become infected when diseased leaves were planted beneath them, while transplanted diseased nursery trees, remote from leaves of the previous year became heavily infected. Primary infections always were correlated with bark lesions containing conidia, and these infections were always found close to the original locus. The areas of infection increased very slowly, producing inverted cone-like diseased regions beneath the original cankers. Under conditions of protracted rainfall, widely scattered single infections were found on leaves and fruit. Copper phosphate mixture 2-4-2-50 throughout the season and lime sulphur 1-50, followed by Bordeaux 1-3-50, gave excellent control of leaf, fruit, and twig lesions. Lime sulphur and Bordeaux, however, caused injury to leaves and fruit, and an early fall defoliation resulted. Copper phosphate mixture caused no injury, defoliation was delayed, and greater production was observed.

An Improved Method for the Preparation of Crystalline Tobacco-mosaic Virus Protein.—
W. M. STANLEY.

The highly infectious dark brown globulin fraction, obtained from extracts of mosaic-diseased Turkish tobacco plants by precipitation with ammonium sulphate, requires further purification before active protein will crystallize. Previously, purification of crude globulin was effected with about a 40% yield of active crystalline protein by the use of lead subacetate and celite. It has now been found possible to prepare crystals with a yield of about 85% by using ammonium sulphate and celite. Purification by the new method was accomplished in 3 different operations: Firstly, about a 1 per cent solution of crude globulin containing sufficient ammonium sulphate (8-11% by weight) to cause a slight turbidity was adjusted to pH 8 and filtered through celite. Secondly, the solution at pH 8 was adjusted to about 20% ammonium sulphate and again filtered through celite. The colored materials remained in the filtrate, while active protein was retained on the celite filter cake. Thirdly, after extraction of the celite filter cake with water at pH 8, the solution obtained was adjusted to pH 4.5 and filtered through celite. Extraction of this filter cake gave an opalescent solution from which active protein was crystallized by the usual procedure.

Viroses of the Garden Pea, Pisum sativum.—MERL W. STUBBS.

Field and greenhouse studies have revealed that more than one virus may cause pea mosaic. Enation pea mosaic, *pea virus 1*, infected soybeans and all pea varieties. Symptoms were dwarfing, foliage spotting, pod distortion, and foliage enations. Pea aphids and plant extract (with or without abrasive) transmitted the virus. Four days aging *in vitro* and 1:3000 dilution inactivated the virus. Marble pea mosaic (*pea virus 2A*), Speckle pea mosaic (*pea virus 2B*), and Mild pea mosaic (*pea virus 2C*) infected white lupine and several pea varieties, excluding Perfection. Marble pea mosaic symptoms were mottling, much foliage chlorosis, dwarfing, leaf-drop, and stem discoloration. Speckle pea mosaic symptoms were speckled mottle, slight chlorosis and other symptoms less severe than those of the first. Mild pea mosaic produced only a very mild mottle. Pod distortion and

foliage enations were not produced by these 3 viruses. The 3 viruses were transmitted by pea aphids and plant extract (only with abrasive). One day aging *in vitro* and 1:1500 dilution inactivated them. All viruses studied infected crimson clover, broad bean, sweet pea, and yellow sweet clover, but none infected red clover or garden bean. No positive evidence of seed transmission was obtained.

Studies on the Winter Injury of Apple Trees.—R. F. SUIT.

Following the severe winter of 1933-34, seven types of injury were found on the apple trees in the Province of Quebec. The prevalence of crotch cankers was associated with the hardiness of the variety, the age of the tree, and the angle of the crotch. The prevalence of branch cankers was influenced by the presence of old pruning wounds, but not by the angle of the branch to the horizontal, nor by the direction of the branch. *Physalospora malorum*, *Schizophyllum commune*, *Cytospora* sp., *Phomopsis mali*, and *Nectria cinnabarina* were found as secondary organisms in crotch and branch cankers. Increasing amounts of black heart were associated with injury to the vessels and xylem parenchyma, xylem rays, cambium, phloem, and cortex, and phloem rays. Black heart of the xylem reduced the flow of water to 10-30% of normal; influenced the efficiency of new xylem formed; reduced the moisture content at mid-season, with an increase to 2.8% above normal by the end of the season; decreased the ability of cuttings to form callus; and increased the electrical conductivity of extracts. Bark splitting was observed in the spring of 1935 and was more prevalent on those varieties affected with black heart.

Further Studies on the Fungicidal Properties of Sulphur.—J. J. TAUBENHAUS AND G. T. BOYD.

Germination of spores of numerous plant pathogens was inhibited by sulphur alone. In certain cases the toxicity of sulphur was increased by adding small amounts of Cuproicide (cuprous oxide) or other copper compounds. In only a few instances, sulphur alone or in combination failed to inhibit spore germination. Thus spores of *Botrytis cinerea* germinated readily on slides dusted with sulphur, copper carbonate, Cuproicide, or Cuproicide + sulphur, although germination was inhibited by thiokolized sulphur. Spores of *Myrothecium roridum* were unaffected by sulphur alone, although germination was inhibited by mixtures of sulphur and Cuproicide or monohydrated copper sulphate. Spores of *Septoria lycopersici* were not affected by sulphur or mixtures of sulphur, Paris Green, Cuproicide, or copper sulphate, but the spores of *Rhizopus nigricans*, *Sclerotinia americana*, *Guignardia bidwelli* (Phyllosticta stage), *Ustilago zeae*, *Gymnosporangium* sp. from cedar apples, *Cylindrocladium scoparium*, *Cladosporium fulvum*, *Actinonema rosae*, *Diplodia* sp. (Macrophoma stage) of the back of roses, *Pestalotzia* sp. of rose canker, *Fusicladium effusum*, sclerotia of *Sclerotium rolfsii* were all inhibited and failed to germinate when dusted with sulphur or sulphur in combination with Cuproicide or other materials.

Growth of Phymatotrichum omnivorum on Normal Roots and on Roots Decayed by Root Rot.—J. J. TAUBENHAUS, W. N. EZEKIEL, AND G. E. ALTSTATT.

It is possible to recover *Phymatotrichum omnivorum* from the recently infected roots of susceptible plants, but not from roots of susceptible plants that have been completely decayed by the disease. In artificial cultures, *P. omnivorum* grew readily on fresh normal cotton or apple roots and on normal cotton roots dried for 3 weeks or longer, but grew poorly on fresh or dried infected cotton or apple roots that had been completely decayed by root rot. Addition of nutrients to completely decayed roots allowed good growth of the fungus. On decoctions from normal cotton or apple roots, *P. omnivorum* grew readily,

while growth was poor on decoctions from cotton or apple roots completely decayed by root rot. By adding nutrients to the decoctions of the completely decayed roots, good growth was obtained. These experiments indicate that the death of *P. omnivorum* on roots completely decayed by root rot is probably in part due simply to exhaustion of nutrients needed by the fungus, particularly carbohydrates, during the course of the decay.

The Dayton Elm Disease.—LEO R. TEHON AND HOMER L. JACOBS.

Since 1928 a dying natural timber and street trees of *Ulmus americana* has been observed in southern Ohio. Severe losses of valuable shade trees have occurred. The syndrome includes droop, wilting, yellowing, and falling of leaves, coupled with cambium discoloration and rotting of small roots and feeders, and cortical disintegration. No symptoms of parasitization appear in aboveground parts. Among 499 attempts at isolation from roots, 318 have remained sterile (owing partly to rigorous technique), 75 have yielded an organism tentatively referred to as *Cephalosporium*, and 106 have yielded in smaller numbers 11 other organisms, of which 39 were *Penicillium* and 20 *Fusarium solani* var. *martii*. The *Cephalosporium* was the only organism consistently isolated from diseased trees. Reinoculations have demonstrated that the *Cephalosporium* will grow on sterile elm twigs, that it will cause dwarfing and death of seedlings grown in test tubes, and that it will cause dwarfing and death of seedlings grown in test tubes, and that it will reproduce the syndrome when implanted in soil about potted elms. The fungus is limited to the phloem, cambium, and young xylem and causes death of the cambium, collapse of the phloem, and, perhaps with secondary invaders, cortical decay.

Fasciation of Sweet Peas. PAUL E. TILFORD.

Since reporting last year that a pathogenic bacterial organism had been isolated from sweet pea plants affected with the disease called fasciation, 15 single bacterial-cell isolates have been obtained, all of which are pathogenic. The morphological and physiological characteristics of these 15 single-cell isolates are essentially identical. A fasciated growth resembling that of affected sweet peas has developed on garden pea, petunia, and gypsophila plants following inoculation. The organism has been isolated from fasciated growths on geranium and chrysanthemum. Soil sterilization and seed treatment seem to be effective control measures. Treating sweet pea seed for 1 minute in alcohol followed by 20 minutes in 1:1000 HgCl₂ is an effective treatment and is noninjurious to the seed.

Dutch Elm Disease in Dead and Dying Elms. R. P. TRUE and EVERETT T. MILLER.

A cooperative Dutch elm disease survey of 750 dead and dying elms was carried on inside and outside of the recognized Dutch elm disease areas in New Jersey, New York, and Connecticut from March to October, 1935. The average number of samples cultured per tree was 15. The trees varied from 3 to 60 inches in diameter at breast height, were from one-half to entirely dead, and were not known to have the Dutch elm disease. The sites of the 27 plots, which contained from 12 to 60, but usually 30 trees each, included swamps, wooded lowlands, open fields with scattered elms, yards, and streets. Within the disease zone the sites were selected to represent areas with varying numbers of confirmed Dutch elm diseased trees per square mile. The average for the 18 plots within the disease area in New Jersey, New York, and Connecticut was 9.9% diseased, while plots outside showed none of the disease. The positive findings indicate the necessity of sanitation work in connection with the Dutch Elm Disease Eradication program. Results of similar, though preliminary, surveys in Cleveland, Indianapolis, and Norfolk were negative. (Division of Forest Pathology, Bureau of Plant Industry, and Dutch Elm Disease Eradi-

cation, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.)

Chlorosis of Rice Induced by Iron Deficiency. E. C. TULLIS and E. M. CRALLEY.

In Arkansas, Louisiana, and Texas chlorosis of rice characterized by (1) bleaching (whitening, not yellowing) of leaf tips and chlorosis of other leaf parts; (2) stunting of individual leaves or entire plants, and (3) reduced yields of affected plants, has been found to be due to an iron deficiency in the plant. Differences in varietal susceptibility to chlorosis are very marked. Most commercial varieties now grown, except Rexoro and Fortuna, are susceptible. (Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station.)

Pathogenicity of Sclerotium rolfsii for Young Apple Trees. THOMAS W. TURNER.

During the summer of 1934 several young apple trees, which had been set out the previous season, wilted and died rather suddenly. The plot had formerly been grown to beans, which from time to time had suffered from severe attack of Southern Blight. Tissue isolations on nutrient agar gave typical sclerotia in about 2 weeks. The fungus, carried over winter on Sabouraud's dextrose agar, was used to inoculate approximately 3-year-old plants of 10 varieties of apples, including Albemarle Pippin, Baldwin, Grimes Golden, Black Twig, Twenty Ounce, Wealthy, Winesap, Winter Banana, York Imperial, and a seedling. They were grown on a selected plot apparently free from this fungus. Inoculum was applied to injured surfaces in some cases, to intact in others, both scion and stock being employed. Almost every inoculated plant became infected as indicated by the extent of the necrotic area, whether inoculated on injured portions or not and whether on stock or scion, but many of them recovered even though the necrotic ring practically girdled the plant. Seventeen of the 44 inoculated plants were dead or dying within 49 days. The controls remained normal.

Hybridization between Sphacelotheca cruenta and Sorosporium reilianum. SYED VAHEEDUDDIN.

A fertile intergeneric hybrid was obtained by crossing monosporidial lines of *Sphacelotheca cruenta* (Kühn) Potter and *Sorosporium reilianum* (Kühn) McAlpine. Five haploid lines of *Sphacelotheca cruenta* and seven of *Sorosporium reilianum* were inoculated hypodermically, singly, and in paired combinations, into sorghum seedlings. None of the haploid lines caused infection, but 11 of 18 paired combinations produced chlorosis on leaves in 8 to 12 days. Normal sori with some characters of *Sphacelotheca cruenta* and some of *Sorosporium reilianum* developed in the inflorescences of some of the chlorotic plants. Different monosporidial combinations of the two species produced sori differing in shape and size. The echinulate hybrid chlamydospores were intermediate in size between both the parents. They germinated readily and produced promycelia, some of which bore sporidia, while others formed numerous hyphal branches but very few or no sporidia. The promycelia and sporidia were significantly larger than those of the parents, possibly due to heterosis.

A Method for Describing Strains of Tobacco Mosaic Virus. W. D. VALLEAU.

Tobacco mosaic viruses collected from tobacco and solanaceous weeds and preserved by drying, may, when tested on a necrotic-spotting and a non-necrotic-spotting variety of tobacco in the greenhouse and on tobacco in the field (for burning), be classified as shown below. Thus a virus may be characterized (but not identified) as necrotic-spotting, non-

burning, distorting, green. At least 9 of the 18 viruses here classified would be recognized as ordinary field mosaic or James Johnson's tobacco viruses 1 or 7. 1. Necrotic-spotting, non-burning, distorting—A. Spots minute: 1. Patterns yellow; 2. Patterns green. B. Spots large: 1. Patterns white. White mosaic, E. M. Johnson; 2. Patterns yellow; 3. Patterns green; 4. Patterns predominantly green, yellow in tomato. English aucuba mosaic; 5. Patterns rings. Ring mosaic, E. M. Johnson; 6. Patterns necrotic rings, in slow-growing plants. 2. Non-necrotic spotting—A. Non-burning: 1. Distorting: a. Patterns white; b. Patterns yellow. Yellow mosaic, E. M. Johnson; c. Patterns green, chlorotic and necrotic rings in Ambalema; d. Patterns green, no symptoms in Ambalema. Severe mosaics 1 and 2, E. M. Johnson; e. Patterns green, less distorting. 2. Non-distorting, mottled: a. Patterns green, several strains. Mild mosaics 1 and 2, E. M. Johnson. 3. Non-distorting, not mottled; a. Faint green rings; b. Yellow rings. B. Burning, distorting: 1. Patterns yellow; 2. Patterns green, several strains.

Resistance of Potato Varieties to Infection by the Veinbanding Virus.—C. L. VINCENT and L. K. JONES.

During the last 12 years, 1055 strains of potato seedlings have been developed and observed relative to susceptibility to infection by the veinbanding virus. All the seedling strains developed from various crosses with the following parents have proved highly susceptible to infection under field conditions: Early Six Weeks, McCormick, Late Rose, Bovee, Early Northern, Keeper, Jersey Red Skin, Irish Cobbler, American Wonder, and Burbank. Many of the strains showed little or no damage from the presence of the virus, but others exhibited extreme symptoms. Of the 420 strains having Katahdin as a parent 22 have shown a high degree of resistance. During 2 years the Katahdin has shown 33 per cent infection and the Chippewa 13 per cent in field plantings compared to 100 per cent infection in a like period of time in Gold Coin, Bliss Triumph, Irish Cobbler, Warba, and U. S. Department of Agriculture strain no. 41956. Extreme necrosis and dwarfing have been associated with the presence of the veinbanding virus in the Warba, Chippewa, and Katahdin varieties.

Resistance to Club Root in Brassica.—J. C. WALKER.

In greenhouse trials with soil from southeastern Wisconsin, naturally infested with *Plasmodiophora brassicae*, the following results were secured. In cabbage, *Brassica oleracea* var. *capitata*, only 3 plants remained free from infection out of a total of 2600 plants from 25 varieties. Shogoin turnip (*B. rapa*) yielded consistently 100% infected plants. Snowball, Purple Top Milan, and White Milan turnips were consistently free from infection. Other varieties of turnip contained varying percentages of infected plants. Most rutabaga, *B. napobrassica*, varieties tested contained small percentages or no infected plants. Great variation in resistance to infection in white mustard (*B. alba*) and in black mustard, *B. nigra*, was found when samples of each from different sections of America and Europe were compared. In each species samples with as low average infection as 16% and others as high as 90% were noted. In a cross between Snowball (resistant) and Shogoin (susceptible) turnip the F_1 hybrid plants were 441 resistant to 22 susceptible.

Histological Changes in Resistant and Susceptible Strains of Maize Infected with Phytonomonas stewartii.—E. J. WELLHAUSEN.

Marked histological differences were found in the reaction of the vascular bundle to bacterial invasion in inbred lines of maize differing in degree of resistance. In certain

moderately susceptible Golden Bantam and white flint lines, infection of the protoxylem stimulated cell division and subsequent lignification in the adjacent parenchyma. A definite radial arrangement of heavily lignified cells around the point of infection was produced. The walls of newly formed tracheae were often pushed in by the pressure of these radially dividing cells. In advanced stages, the attempted formation of new tracheids at various points in this lignified area was evident. While this apparent walling off of the first point of infection was taking place, the tracheae and tracheids gradually became plugged, resulting in slow death of the plant. In very susceptible lines, rapid plugging of the xylem vessels and deterioration of the thin-wall parenchyma cells occurred. The entire bundle, including the phloem, was destroyed in extreme cases. Apparently, invasion was so rapid that destruction occurred before a counteraction of the host could be brought about. These lines completely wilted shortly after inoculation. In highly resistant lines the morphology of the bundle was not changed. Relatively few bundles showed infection, with seldom more than one or two vessels plugged or partly plugged.

Parasitism of the Apple Leaf Hopper, Typhlocyba pomaria, by Entomophthora.—S. A. WINGARD.

The leaf hopper, *Typhlocyba pomaria*, occurred in injurious numbers in Virginia apple orchards the latter part of August, 1935, and some of the growers prepared to spray for its control. Spraying, however, was prevented by almost continuous rainfall during the first week of September, after which the leaf-hopper infestation seemed to be much less severe. Upon examination, many dead leaf hoppers were found adhering to the undersurface of the apple leaves. Examination revealed that the dead leaf hoppers had been parasitized by a fungus, which, from the morphological characters of its hyphae and spores, appears to be *Entomophthora sphaerosperma*.

Some Pathological Problems in Connection with the Development of the Plains Shelter-belt Project.—ERNEST WRIGHT.

A branch of the Division of Forest Pathology, United States Bureau of Plant Industry, has been established at Lincoln, Nebraska, in cooperation with the United States Forest Service and Plains Shelter-belt Project. Confronting problems may be of general interest. The numerous species grown, diversity of soil types, and climatic factors, render nursery problems particularly interesting. Root rot and damping off of young deciduous seedlings have been surprisingly common, American and Chinese elm being most universally infected. Control through seed or soil treatments appears necessary for these species. Leaf blights of older nursery stock may require spraying for control in some cases and wilts will be controlled through frequent elimination. Seedling storage problems also are important, especially the Chinese elm root rot caused by *Chalaropsis thielavioides*. Diseases have not yet caused serious losses in recently planted shelter belts. Pressing problems have been to map, before planting, the areas within the southern end of the shelter-belt zone that are infected with Texas root rot. An attempt may be made to locate in advance those areas where noninfectious chlorosis is common. Nematode damage also may prove common in certain areas. With the aid of advanced surveys it is hoped many undesirable areas may be avoided or resistant hosts employed.

Downy Mildew of Hops. CECIL E. YARWOOD.

Fall and spring applications of granular calcium cyanamid and of copper sulphate-lime dust to dormant hop hills caused no significant reduction in the number of systemically infected shoots (spikes) arising from these hills in 1935. Weekly counts of the

number of spikes appearing in a total of 1,860 hills during the spring months indicated that most spike infections were of secondary origin. Under field conditions with dry days and humid nights, there was a regular diurnal cycle of the causative organism *Pseudo-peronospora humuli*. Sporangioophores and sporangia were formed during the night, the sporangia were matured in the early morning after dawn and liberated during the day. The alternation of light and darkness is necessary for the expression of this diurnal cycle, and sporangia were not formed when mildewed leaves were subjected to light and high humidity at night. Field and laboratory applications of fungicides were of value in the following order: 0.5% Bordeaux, 1% zinc lime, 1% rosin soap, 1% Palustrex (containing 0.2% copper resinate), 0.25% Coposil, and 0.25% cuproside. Laboratory studies indicate that differences in the spreading qualities of spray materials cause differences in the amount of spray deposit on the leaves and influence the fungicidal efficiency of these materials.

A Pea Streak Caused by Alfalfa Mosaic.—W. J. ZAUMEYER AND B. L. WADE.

An alfalfa mosaic virus collected at Rosslyn, Virginia, was shown by cross-inoculation studies to be infectious to pea, *Pisum sativum*, producing a decided streaking of the stems, petioles, and leaves. The disease is readily transmitted by the usual mechanical means of rubbing the virus extract on to healthy leaves. Infection was noted 13–15 days after inoculation. The first symptom to appear is a slight purpling and streaking of the stems, a recurving of the stipules, and downward curling and distortion of the leaflets. The streaking may later extend from the base to the tip of the plant. The veins and veinlets of the leaves are slightly cleared at first but later become reddish brown. The apical leaves often manifest a rosetted condition. Infected plants show wilting and often die. The phloem tissue becomes discolored and necrotic. Pods formed before the plants become seriously infected, take on a dark purplish gray discoloration. They may be spotted and pitted and decidedly malformed, and frequently do not reach maturity. of 38 varieties of peas tested, no resistance was shown by any. The disease has not been reported wide-spread under field conditions. It apparently is not identical with the pea streak described by Linford in Hawaii.

Pea Mosaic and its Relationship to Other Legume Viruses.—W. J. ZAUMEYER AND B. L. WADE.

Field surveys showed pea mosaic usually more severe along borders of fields than elsewhere, suggesting the possibility of the transmission of certain legume viruses to pea. Cross-inoculation studies showed that the mosaic viruses of red clover, white clover, white sweetclover, and alsike clover are infectious to pea. It is probable that other mosaic viruses may be found in the several legumes that may react differently from those described, in spite of symptom similarity. Forty-two varieties of peas were tested for resistance and susceptibility with the several viruses. Of a total of 3057 seeds collected from mosaic-infected plants, 11 produced diseased seedlings. All infected seedlings were produced from seed collected from above the point of noticeable infection. In addition to the production of symptomatological differences on certain pea varieties, the several viruses can be separated on the bases of the reaction of Stringless Green Refugee, Corbett Refugee, Robust, and Resistant Great Northern beans, and of *Vicia faba*. It is believed that mosaic-infected legumes play an important part and are responsible for much of the disease present in the pea crop. Their importance in relation to the spread of the several viruses to pea is that they offer a continuous source of infection.

THE ANTHRACNOSE OF CURRANT AND GOOSEBERRY CAUSED BY PSEUDOPEZIZA RIBIS¹

E. C. BLODGETT²

(Accepted for publication February 9, 1935)

Anthracnose of currant and gooseberry caused by *Pseudopeziza ribis* Kleb. has been the subject of numerous investigations in Europe and America for over 45 years. Frequent notes on the occurrence and severity of the disease have appeared and numerous efforts have been made to develop control measures. However, the disease has received little critical attention since the valuable work of Klebahn (18) in 1906. The primary objects of the present paper are to provide a survey of the literature, to record certain studies on the physiology and life history of the fungus, and to evaluate certain factors important in the epidemiology and control of the disease.

THE DISEASE

The occurrence of the disease in Europe on *Ribes rubrum* L. was reported in 1867 (17), and 6 years later it was first reported in America by Berkeley (1) on the cultivated black currant, *Ribes nigrum* L. Although the disease was regarded by Dudley (8) in 1889 as becoming important in the United States, it apparently attracted little critical attention either in America or in Europe until about 1899, when it became serious. Klebahn (18) discovered the ascigerous stage and named the fungus *Pseudopeziza ribis* in 1906. Although the disease was reported by Carruthers (4) in England as early as 1903 and undoubtedly was present before, it did not become of great importance there until about 1924, as pointed out by Briton-Jones (2).

The distribution of anthracnose is almost world-wide with the cultivation of the hosts. It has been reported from most of the countries of Europe from Norway to Italy, from Asia, New Zealand, Australia, every Province in Canada, from Alaska, Mexico, and the following States of this country: California, Colorado, Connecticut, Delaware, Idaho, Illinois, Indiana, Iowa, Massachusetts, Michigan, Minnesota, Missouri, Montana, New Hampshire, New Jersey, New York, North Dakota, Ohio, Oregon, Pennsylvania, South Dakota, Vermont, Virginia, Washington, and Wisconsin.

¹ Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

² The writer wishes to express his special appreciation to Dr. G. W. Keitt, under whose direction this study was made, for helpful advice and kindly criticism throughout. He is also grateful to Dr. E. M. Gilbert for help in histological studies and to Dr. J. J. Davis for identifying the fungus. Thanks are due to Mr. Eugene Herrling for the photographic work.

The importance of the disease has been largely underestimated because of the nature of the injury. Direct losses may reach 75 per cent of the crop through fruit infection, defoliation, and subsequent sunscald of the fruit. Furthermore, the plants may be so reduced in vitality, and hence rendered more susceptible, that successive crops may be reduced by 50 to 80 per cent. The evidence presented by Ewert (11) and Müller (27), and confirmed during the present study, although no data are included here, show that fruit from bushes severely attacked by the fungus is low in sugar and very poor in quality. Anthracnose is considered the most widespread and destructive disease of cultivated currant and gooseberry. While not of direct economic importance on wild *Ribes* spp., it is a very serious pest on these plants.

The susceptibles of *Pseudopeziza ribis* include approximately 25 species of *Ribes* plants of diverse nature and wide distribution. The list includes all common species of both currant and gooseberry and many wild ones, but no host outside the genus *Ribes* has been reported. One of the limiting factors in either a study of or report on susceptibility of both species and varieties is the extremely confused state of nomenclature of *Ribes* plants. This fact has prevented the writer from including here a list of reported susceptibles because it would only add to the confusion.

The symptoms of anthracnose on *Ribes* vary under different conditions and more especially on different hosts. The fungus may attack all except woody portions of the plant above ground, but injury is most common on the leaves (Plate I, A, B, F). Here the manifestations of the disease first appear as small discolored areas that later enlarge and often coalesce, forming large brown patches visible on either surface. Depending on the species or variety affected, the type of lesion varies greatly; but, characteristically, it is dark brown, one to several mm. in diameter, circular or angular, and sharply delimited, though often with a light yellow border. With the first appearance of the disease, or shortly after, the leaves may turn yellow, quite commonly leaving a green zone around individual lesions. On certain wild species of *Ribes* and some cultivated gooseberries, the spotting is often accompanied by brilliant reddish or purplish pigmentation of the surrounding tissue. Typically the fungus attacks the older foliage first and especially that of bushes in poor vigor or of varieties naturally weak in growth. Premature defoliation, which may be complete by the middle of July, always is associated with anthracnose. With very severe infection, the leaves may turn brown, wither and fall, as if scorched. There is, however, no indication that the mycelium from a single lesion may infect the whole leaf and finally the entire plant, as reported by Laubert (21). Several acervuli may develop on each old lesion. Here masses of conidia, bound in a water-soluble slime, appear as white tendrils or dull translucent drops (Plate I, D). Generally

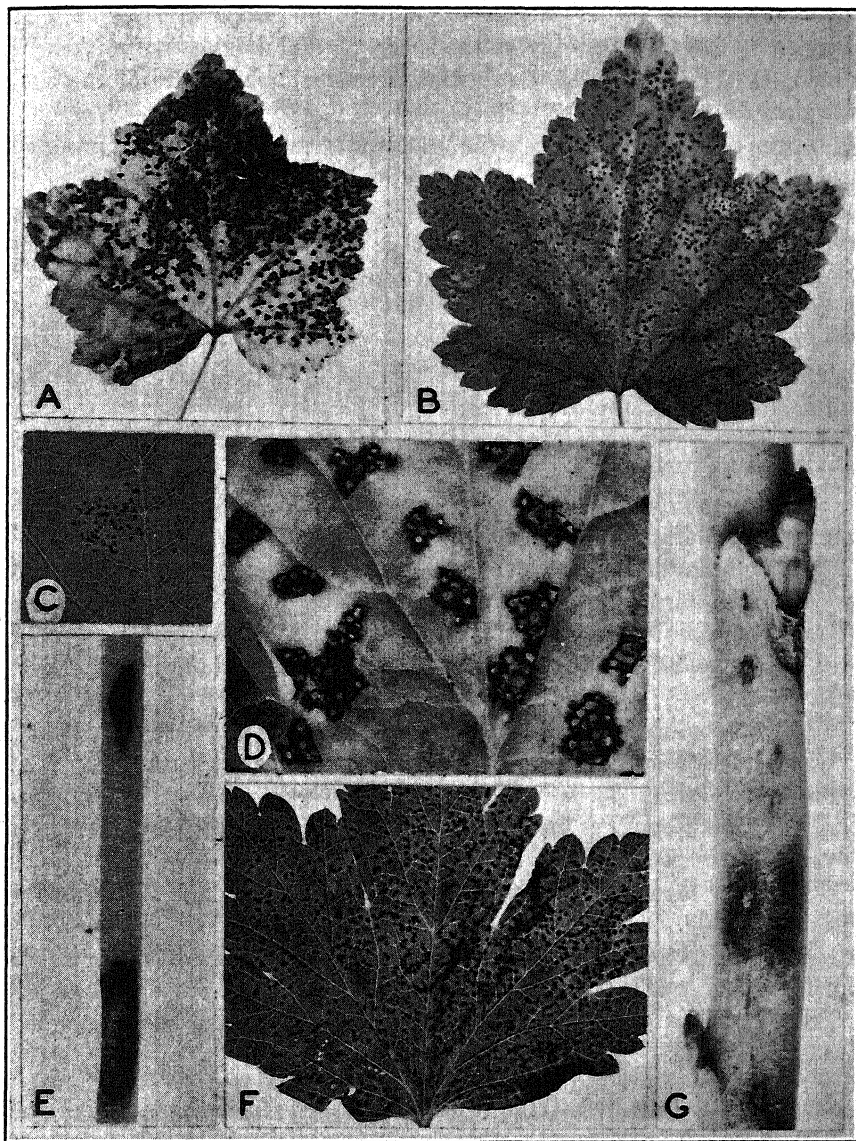


PLATE I. Symptoms induced by inoculations of currant and gooseberry with *Pseudopeziza ribis*. A. Red Cross leaf inoculated with conidia from currant, showing severe type of infection. Slightly reduced. B. Diploma leaf inoculated with ascospores from currant. Slightly reduced. C. Portion of Diploma leaf inoculated with ascospores from currant, using a special technique to limit infection to a certain area. $\times 1$. D. Red Jacket leaf inoculated with conidia from gooseberry, showing lesions produced and abundant sporulation. $\times 3$. E. Red Jacket petiole inoculated with ascospores from gooseberry. $\times 4$. F. Red Jacket leaf inoculated with ascospores from gooseberry. Slightly enlarged. G. Red Jacket petiole inoculated with ascospores from gooseberry. Slightly enlarged.

these form on the upper surface of the leaf, but in some cases they may occur on both surfaces or only on the lower. Final diagnosis of anthracnose is made by the presence of the typical crescent-shape conidia of the causal organism (Plate II, A-G).

On the petioles and pedicels the lesions may girdle the organ, producing a small canker (Plate I, E). The symptoms on the fruit appear as tiny black specks, but the attack may cause severe shelling of the fruit, as reported by Clinton (5) and shown in his Plate IVa. On the young nonwoody shoots the lesions appear as superficial golden brown areas (Plate I, G).

THE CAUSAL ORGANISM

Eleven isolates of the fungus have been used in this study. Their origin is indicated in the following notes:

1. From conidia on leaves of either Cherry currant or of gooseberry, Sturgeon Bay, Wis., July, 1931.

2. From conidia on cultivated red-currant leaves sent through the courtesy of H. P. Barss, from Corvallis, Oregon, July, 1931.

3. From conidia on cultivated red-currant leaves sent through the courtesy of W. L. Gordon, from Winnipeg, Manitoba, Sept., 1931.

4. From conidia on Poorman leaves, Madison, Wis., Oct., 1931.

5. From conidia on cultivated red-currant leaves, Madison, Wis., Nov., 1931.

6. From conidia on Franco-German leaves, Madison, Wis., Oct., 1932.

7. Culture from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, Jan., 1933.

- 1 C. From ascospores on overwintered Cherry currant leaves, Sturgeon Bay, Wis., May, 1933.

- 1 G. From ascospores on overwintered gooseberry leaves, Sturgeon Bay, Wis., May, 1933.

- 4 G. From ascospores on overwintered gooseberry leaves, Madison, Wis., April, 1933.

- 6 C. From ascospores on overwintered Franco-German leaves, Madison, Wis., April, 1933.

Some isolations were extremely difficult to make from the material available, because the fungus grows very slowly (especially isolates 3 and 5) and occasionally certain lots of spores, for no determined reason, failed to germinate. These difficulties have been mentioned previously by Ewert (11), Klebahn (18), and Luijk (23). Spore masses, naturally formed or forced out on leaves in a moist chamber, were removed with a sterile needle to nutrient agar and, later, pure culture transfers were made. Ascospores were allowed to germinate after being discharged (on nutrient agar) from

portions of leaf bearing ascocarps. Single-spore cultures were secured from isolates 1 to 4, inclusive, but only after many failures in the case of No. 3. Numerous attempts were made with spores of isolate 5 but without success. All of the studies reported in the case of isolates 1-4 refer to the single-spore cultures. Stock cultures were maintained on potato-dextrose agar kept in the ice-box or at room temperature.

The identification of the fungus as *Pseudopeziza ribis* from the leaf specimens mentioned above was done by the writer and confirmed by Dr. J. J. Davis.

The morphology of *Pseudopeziza ribis* cannot be treated adequately within the scope of this paper, so mention is made only of pertinent facts, and the reader is referred to Laubert (21), Klebahn (18), Janczewski and Namyslowski (14), and Luijk (23). An examination of the conidia produced by the different isolates in culture showed consistent variation in size and shape of the spores. In view of the usual disagreement of workers on the size and shape of conidia of *Pseudopeziza ribis* it seemed advisable to determine the question for the isolates concerned in this study. One hundred spores from the original leaf material for each isolate were measured as usual, for width taking the maximum diameter and for length taking the shortest distance between the most extreme parts of the spore regardless of curvature. Then, for each isolate, 400 spores taken from colonies grown on potato-dextrose agar at 20-24° C.³ were measured as above. Since these values did not compensate for the curvature of the spore, a suspension of representative conidia from culture was placed on a clear agar medium and photographed. The photograph was then projected onto paper and the outline of the spores drawn. Using a ruler and a rather stiff copper wire, 2 measurements were taken: first, the same measurement as above and, second, the length of the line from end to end equidistant from the sides of the spore. By proportion the corrected or actual length of the conidia in culture was computed. The results of these measurements and comparisons are shown in table 1 and by the photographs in plate II, A-G. The data indicate that the spores in culture are much larger than those produced on the host.

To determine the effect of different hosts on the spore size of one strain, the following experiment was performed. A Red Jacket plant was inoculated in February, 1934, with ascospores from gooseberry leaves from Sturgeon Bay, 1933. When conidia were available on this plant they were used in inoculating 3 varieties of currants and 2 of gooseberries and all bushes were kept under the same conditions. After the lesions were well formed the leaves were placed in a moist chamber and after sporulation, 50 conidia were measured as usual. The results showed only slight differences in size

³ Temperature is expressed throughout this paper in degrees Centigrade.

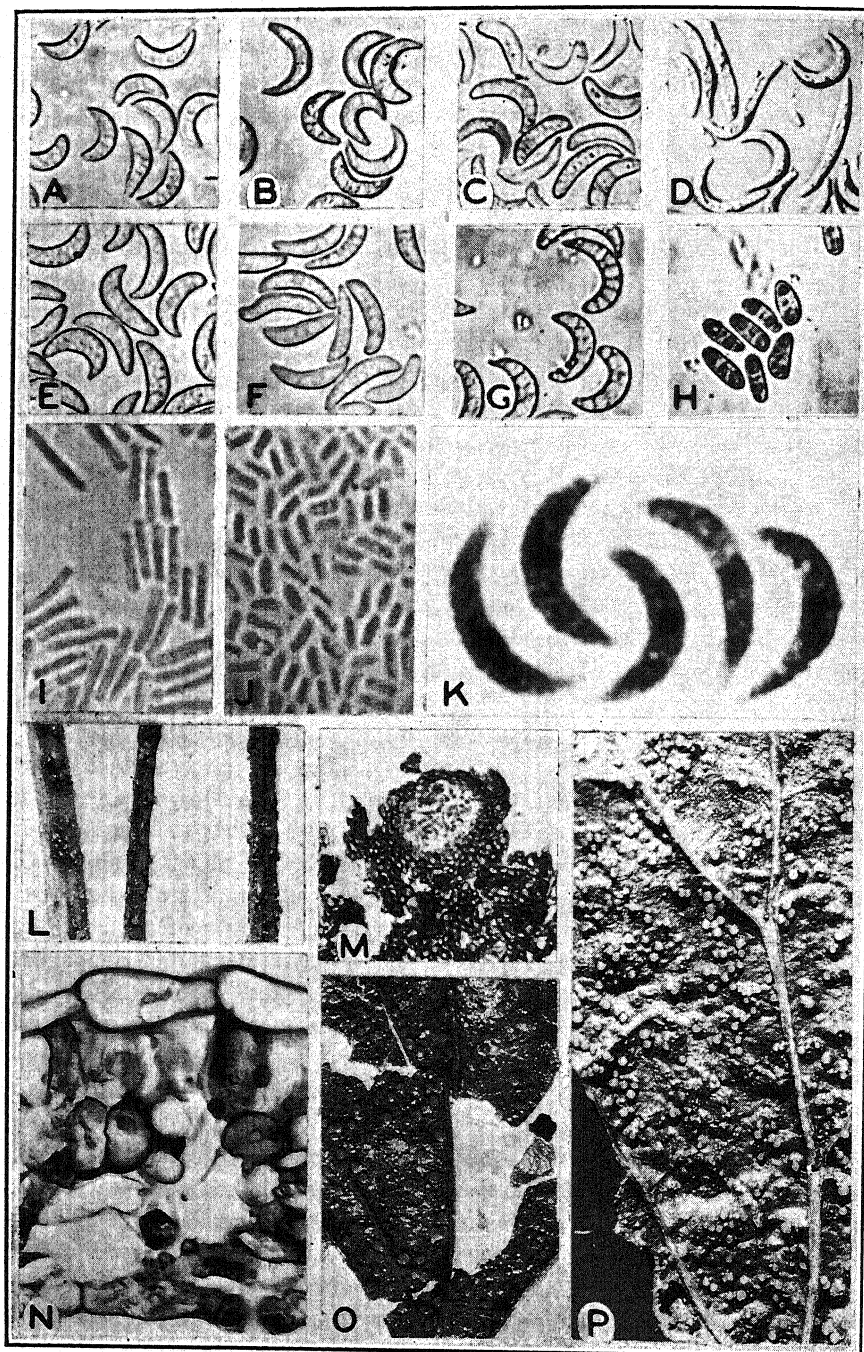


PLATE II

and spore shape. The range was 20.5 to 23.0 microns in average length on Diploma and Cherry, respectively, and 5.3 to 5.9 in average width on Wilder and Downing, respectively. Conidial fruiting was sparse in some cases, however, sometimes accompanied by the production of microconidia.

The effect of temperature on size of spores was determined by measuring 30 conidia in each of 2 tests with leaf material incubated at temperatures of 4–32° at 4° intervals. As a check on these results, 50 spores at 4, 12, 16, 20, and 28° and 100 spores at 8 and 24° produced by isolate 3 growing on potato-dextrose agar for 12 weeks were measured as usual. These results indicated that there was little difference in size and shape of spores produced at the different temperatures. The range in average length was from 19.8 μ at both 20° and 32° to 22.5 μ at 22°; and in average width from 5.0 μ at 20° to 6.0 μ at 4°, 8°, 22°, 28°. Conidia in culture, however, were larger than those found on the leaves and varied in average length from 25.2 μ at 28° to 29.7 μ at 24°; and in average width from 5.8 μ at 28° to 6.7 μ at 16°.

Conidia have granular or extremely vacuolate contents and, although very few spores in nature possess a cross wall (until germination), such walls are common in culture. Spores fixed in very dilute Flemming's weak fluid and stained with Delafield's haematoxylin show the presence of one nucleus (Plate II, K).

Microconidia in cultures of *Pseudopeziza ribis* were first described by Klebahn (18), but he was very cautious about admitting their connection with the fungus under study. Janeczewski and Namyslowski (14), found them in abundance on the host. These spores in nature as in the case of conidia vary greatly in size. They are usually rod-shape with rounded or quite blunt ends. Some appear uniseptate, but the identity of the wall is uncertain. They are granular or vacuolate, hyaline, and appear to be uninucleate. Microconidia from isolate 2 grown on green bean pods in test tubes were 8.9 \times 2.3 μ in size, as shown by an average of 400 measurements. A comparison of microconidia from the 7 isolates grown on potato-dextrose agar at 20° for 8 weeks shows 2 distinct classes. Isolate 2 forms large microconidia 11.2 \times 1.6 μ (Plate II, I) and the other 6 isolates small ones,

PLATE II. A–G. Unstained conidia of isolates 1–7, respectively, from potato-dextrose agar. $\times 350$. H. Unstained ascospores from gooseberry leaves. $\times 335$. I and J. Unstained microconidia of isolates 2 and 4, respectively, from potato-dextrose agar. $\times 850$. K. Conidia of isolate 1 stained with Delafield's haematoxylin, showing uninucleate condition. $\times 1180$. L. Ascocarps on overwintered Franco-German petioles. $\times 3$. M. Overwintered Franco-German leaf, showing conidia borne in a pycnidium-like body. $\times 165$. N. Downing gooseberry leaf, showing the primary hypha after penetration by the fungus. Further development has occurred involving the entire area shown, with many cells completely filled with mycelium and some already killed. $\times 500$. O. Fragment of overwintered gooseberry leaf covered with mature ascocarps. $\times 3$. P. Fragment of Franco-German leaf covered with mature ascocarps. $\times 3$.

TABLE 1.—Size of conidia of the different isolates of *Pseudopeziza ribis* produced respectively on the leaf and in culture

Source of spores	Measurements in microns from stated isolates: ^a						
	1	2	3	4	5	6	7
Gooseberry leaf	22.6 × 5.3	20.5 × 5.7	24.9 × 5.5
Current leaf	18.9 × 5.4	17.1 × 5.8	18.0 × 5.3 ^b	18.0 × 5.7
Spores from colonies on potato dextrose agar at 20–24°	21.6 × 6.7	23.1 × 7.0	27.1 × 7.0	25.8 × 6.4	25.7 × 7.2	27.4 × 7.3	27.3 × 7.1
Do. (Actual length of spores) ^a	28.1	33.9	33.9	32.8	32.0	30.2	36.8

^a See text for methods used and number of measurements.

^b These leaves were collected in the same isolated garden the following year. In all other cases the leaf material represents the source of the original isolate. See notes earlier in the paper.

those from isolate 4 (Plate II, J) measuring $4.8 \times 1.4 \mu$, each measurement being an average from 100 spores. The rôle of these small bodies in the life history of the fungus is unknown, but it is probably in connection with fertilization, as demonstrated recently in other fungi by Dodge (6), Drayton (7) and others.

Klebahn (18, 19) has reported the perfect stage of *Pseudopeziza ribis* on *Ribes rubrum* and *R. nigrum*, but considers (20) that the fungus on *R. grossularia* has lost its power of producing apothecia. The writer has found ascocarps of the fungus on *R. rubrum*, on the wild black currant (*R. americanum* Mill.) and on *R. grossularia*. Rather wide variations occur in the size and shape of the ascocarps on both currant and gooseberry, but no characteristics were consistent enough to distinguish the fruiting bodies on the different species (Plate III, H, I). The average measurements of 500 ascospores from each *R. rubrum* (Franco-German) and *R. grossularia* (cultivated) were, respectively, 15.5×6.7 and $20.2 \times 9.3 \mu$. The ascospores on currant were matured at 12° – 16° during April, 1933, at Madison and after being discharged on 1.7 per cent agar in water were preserved in 70 per cent alcohol. Those on gooseberry matured in nature at Sturgeon Bay and were measured after being discharged on 1.7 per cent agar in water.

PHYSIOLOGICAL STUDIES

Sporulation. Conidia may be present on leaves at the first macroscopic evidence of the disease and continue to form as long as the leaves remain on the bush. A moist atmosphere greatly accelerates spore production. The effect of temperature on sporulation of the fungus on gooseberry leaves was determined by 3 experiments. Leaves of Red Jacket bushes, artificially inoculated in March, 1933, and kept in the greenhouse, were picked on April 27 and, after being washed to remove nearly all the spores, were placed in moist Petri dishes and held at temperatures from 4° to 32° at 4° intervals. After 48 hours, records were taken by rating the leaves in regard to abundance and size of spore masses (Plate I, D). This experiment was repeated in February, 1934, using Downing leaves with smaller lesions and extending the temperature range from about 1° to 36° . Another test in March, 1934, was conducted similarly, using Downing leaves that were thoroughly brushed and washed to remove essentially every spore. In the last test on leaves below 8° and above 28° very few spores were formed; indeed, at more favorable temperatures they were sparingly produced. It is considered that injury to the acervuli in brushing accounts for the difference from the results of the earlier experiments. As a result of these tests it is concluded that sporulation will occur at any temperature generally tolerated by the growing host, the optimum falling between 20° and 28° . It also was found that spores produced at temperatures from 4° to 32° would germinate on

agar at 16°. Only abnormal spores were formed at 36° and none of these tested would germinate. Field observations and tests indicate that viable spores are present even during extremely hot dry periods of the summer.

In pure culture the fungus usually produced conidia abundantly, the several isolates, however, behaving very differently. White glistening drop-like masses composed of spores were formed in all isolates except No. 7. Age of the culture at which sporulation occurred varied widely, isolates 1, 2 and 4 fruiting first and 3 and 5 much later. Of the media used, sterile green bean pods and potato-dextrose agar were most conducive to fruiting of the fungus. The colonies grown on potato-dextrose agar in 6-oz. bottles in the temperature series of 1933 and 1934 were examined and the temperature curve for sporulation secured. The 2 colonies in each bottle were scraped with a wire hook and water suspensions made on glass slides. Three bottles of each isolate at each temperature for the 2 series were examined at the end of 8 weeks. The abundance of conidia and microconidia was rated in values from 1 to 7. These are averaged and shown in table 2. The results indicate that the optimum temperature for conidial production of the fungus

TABLE 2.—*Relation of temperature to the production of conidia and microconidia of Pseudopeziza ribis on potato dextrose agar*^a

Temperature	Relative abundance of spores produced by stated isolates:							
	1	2	3	4	5	6	7	Av.
Conidia								
4°1	.2	.8	0 ^b	.7	.2	.1	.3
81	.2	1.4	0	.5	1.0	.2	.5
12	0	.2	2.4	0	.2	.2	.4	.5
162	.2	3.8	.2	3.2	2.2	.7	1.5
20	3.7	1.3	5.8	1.7	7.0	7.0	3.2	4.2
24	5.8	1.7	6.8	7.0	4.7	7.0	4.7	5.4
28	3.5	.8	.8	6.8	.4	1.3	2.5	2.3
32 ^c
Microconidia								
4°	2.6	1.9	.3	4.2	.8	.9	.7	1.6
8	5.0	5.7	.3	4.7	1.0	2.3	2.3	3.0
12	6.8	7.0	.4	6.2	1.1	2.3	1.7	3.6
16	6.8	7.0	.8	5.5	1.1	3.0	1.0	3.6
20	6.3	3.3	.3	5.2	.3	.4	1.0	2.4
24	2.1	2.5	.3	3.3	.5	0	1.1	1.4
284	.6	.1	1.3	.3	0	1.1	.5
32

^a See text for conditions of the experiment.

^b No spores produced.

^c No growth of the fungus.

under these conditions is about 20°–24°. Under these same conditions temperatures of 8°–16° were more favorable for the production of microconidia (Table 2).

Similar tests were made on colonies of the fungus growing in 12-oz. bottles at 20°–22° for 8 weeks on a special medium at different pH concentrations. One bottle of each of the 3 series was examined for each isolate at each pH and the records are averaged and shown in table 3. From these data it appears that conidial production was greatest under these conditions at about pH 4 and microconidial production was abundant whenever growth occurred.

TABLE 3.—*Relation of pH of the medium to the production of conidia and microconidia of Pseudopeziza ribis*^a

Isolate	Relative abundance of spores produced at stated pH:							
	3.02	3.98	4.98	5.40	5.96	6.97	8.12	9 ±
Conidia								
1	0 ^b	4.7	.7	1.7	2.0	1.3	1.0
2	0	3.7	3.3	6.3	4.3	2.0	1.3
3 ^c	.7	.7	0	0	0	0
4	3.3	3.3	5.3	5.3	6.7	2.3
5	2.0	1.3	1.0	.3	1.0	0
6	6.3	6.7	4.0	2.3	.3	0
75	.5	0	0	0	0
Av.	0	3.2	2.4	2.6	2.0	1.6	.7
Microconidia								
1	0	6.3	7.0	7.0	7.0	7.0	2.3
2	0	5.7	6.7	5.0	5.3	5.0	7.0
3	1.3	2.7	2.3	2.0	2.0	2.7
4	7.0	5.3	5.3	3.0	7.0	6.3
5	2.0	2.7	2.3	4.0	2.0	4.7
6	5.0	1.7	1.0	.7	1.0	1.0
7	2.5	1.7	5.0	5.0	6.7	6.0
Av.	0	4.3	4.0	4.0	3.9	4.4	4.3

^a See text for conditions of the experiment.

^b No spores produced.

^c No growth of the fungus.

Microconidia may be formed in nature at any time during the summer but most abundantly in the latter part of the season. They also may appear in great numbers along with conidia on overwintered leaves bearing ascocarps. Artificially, they are produced more commonly on old cultures or under adverse nutrient or environmental conditions.

Ascospores are produced in apothecia borne in the spring on overwintered leaves and petioles (Plate II, L, O, P). No ascospores have been seen in artificial cultures, although bodies suggestive of perfect fruiting structures are frequent on the mycelium formed in a "secondary growth" of old cultures. This rapidly growing mycelium later becomes dark, with thick walls, and appears much like that formed in leaves during the fall and early winter.

Germination. Ascospores germinate readily in drops of water, on 1.7 per cent agar in water, and on potato-dextrose agar. The germ tube apparently may arise at any place on the surface of the spore, producing in some cases a rather large appressorium and a thick stocky germ tube that generally branches profusely and forms a dense mass of septate hyphae. Germination may begin in a few hours but proceeds slowly. Klebahn (18) figures ascospores within the ascus germinating and producing conidia.

Reports on the germination of conidia have resulted in contradictory evidence. Ewert (11) reported after numerous trials that spores would germinate only after the fall frosts. Luijk (23) found that spores from *Ribes nigrum* would not germinate in July or after storage for a month in the laboratory. In August the form of *R. grossularia* germinated readily. The writer has found that spores, both in nature and in pure culture, may germinate, or they may not, any time after they are formed. These facts have presented great difficulties some of which have been overcome, but the reasons for such behavior have not been formulated. It will thus be seen that the selection of spores for germination tests has been at best somewhat unsatisfactory. However, all precautions possible were taken to use only comparable material for tests reported herein. In most cases spores were taken from colonies on potato-dextrose agar or on bean pods by removing the spore horns with a wire hook and making a suspension in sterile distilled water. Preliminary trials indicate that spores may germinate in all concentrations of suspensions from a few per low-power field to many hundreds, the advantage, in general, being with the medium to heavy concentrations. The germ tube may arise anywhere on the surface of the spore, though generally near the end. Frequently, during germination, the spore becomes uni- or multiseptate. Spores of isolate 5 produce relatively thick, compact germ tubes, while those of isolate 4 send out thin, thread-like germ tubes. Appressoria may be produced.

Temperature Relations. The effects of temperature and of 2 common media on the germination of conidia at different time intervals are shown in table 4. Although these data were secured by a single test in duplicate involving generally 100 or more spores, they are representative of results from several series of similar experiments using spores from the same and other sources. Conidia were placed in sterile, double-distilled water, and

TABLE 4.—*Relation of temperature and of two different media to the germination of spores of Pseudopeziza ribis*

Isolate	Spores and medium ^a	Hours	Per cent germination at stated temperatures:							
			4°	8°	12°	16°	20°	24°	28°	32°
1	Conidia Double distilled water	24	0	0	0	8.9	21.5	4.2	0	0
		48	0	2.8	52.6	42.9	34.8	13.0	0	0
		96	0	22.2	57.9	43.3	21.8	0	0
2	Do.	24	1.4	7.2	7.0	23.3	10.0	12.1	8.3	0
		48	7.4	22.8	15.7	24.8	18.9	26.3	8.3	0
		96	36.5	38.4	31.0	26.6	35.5	28.2	9.0	0
3	Do.	24	0	0	0	0	0	0	0	0
		48	0	0	0	0	0	0	0	0
		96	0	0	0	0	2.9	0	0	0
4	Do.	24	4.7	6.0	14.3	40.3	42.3	39.6	2.9	0
		48	18.7	27.3	47.6	43.0	42.4	50.0	16.9	0
		96	19.6	44.8	66.0	79.2	65.7	57.3	41.3	0
5	Do.	24	0	0	0	0	0	0	0	0
		48	0	0	0	2.1	0	0	0	0
		96	0	0	0	4.0	0	0	0	0
1	Conidia 1.7% agar in water	24	9.6	13.6	48.7	69.1	72.4	54.4	19.8	0
		48	39.5	38.8	76.3	83.8	89.0	86.2	27.4	0
		96	78.3	77.8	80.3	87.5	93.2	31.3	0
2	Do.	24	15.4	21.8	48.5	78.8	79.4	82.6	64.1	0
		48	50.7	52.2	71.2	82.9	95.4	90.8	74.6	0
		96	76.2	71.2	81.3	88.2	0
3	Do.	24	0	0	0	0	0	0	0	0
		48	2.1	11.5	5.0	35.0	23.8	0	0	0
		96	55.6	75.0	76.7	88.9	59.6	4.8	0	0
4	Do.	24	27.0	27.5	44.1	75.7	93.0	92.9	78.9	0
		48	52.4	61.7	74.7	86.9	95.1	94.7	91.6	0
		96	84.4	70.8	78.1	0
5	Do.	24	0	3.2	24.6	35.1	20.5	20.3	0	0
		48	48.6	39.7	73.4	68.4	68.2	33.8	0	0
		96	66.7	71.7	88.0	91.1	86.8	55.4	3.2	0
	Ascospores 1.7% agar in water	40	76.0	83.3	81.2	75.5	49.3	36.7	30.2	0

^a Conidia in hanging drops. All conidia from fungus grown on bean pods at 20° for about 30 days. Ascospores from overwintered Franco-German leaves.

hanging drops were made, using chemically cleaned glassware. Drops of this suspension were placed also on 1.7 per cent agar in water in Petri dishes and then all spores were incubated at the different temperatures. Besides the readings on germination, the lengths of the germ tubes were measured after 48 hours at the various temperatures and are as follows for spores at 20°:

Isolate	In double-distilled water	On 1.7% agar in water
1	84 microns	210 microns
2	56 do	210 do
3	0 do	14 do
4	126 do	266 do
5	0 do	42 do

The differences shown by the several isolates in both germination and early growth are very great and are typical of their behavior throughout the period of investigation.

To determine the effect of temperature on discharge of ascospores, the following technique was employed for the tests in 1933. Two pieces of current leaf bearing mature ascocarps were moistened on the under side of a Petri dish lid so that the spores would shoot down onto a film of 1.7 per cent agar in water. The areas were marked and the plates let stand at room temperature for about a half hour to see that spores were discharged in all cases. Then 2 plates were placed in each incubator at temperatures of 4°–32° at 4° intervals and 2 plates were set on ice. All were left for 30–45 minutes and then the lids of the dishes were moved over a new area marked 2. After 48 hours an examination showed that spores had been discharged at all temperatures but most abundantly at 16°–24°. This experiment was repeated, using another technique. The leaf material was dried down at room temperature, and, after it was placed in the incubators for 45 minutes, a new area was marked and the leaf tissue moistened with water from a medicine dropper that had come to the temperature of the incubator. The results confirmed those of the first series.

A similar procedure was used in securing ascospores for germination tests at different temperatures. Spores were discharged in Petri dishes on 1.7 per cent agar in water over a period of about 1 to 1½ hours at room temperature and then the spores were incubated at 4°–32° and on ice. The results of 2 such series are averaged and shown at the bottom of table 4.

Growth of the fungus at different temperatures was compared in an extensive trial in 1933 and repeated in 1934. Twenty-five-cc. portions of potato-dextrose agar were placed in 6-oz. glass bottles stoppered with cotton. After the agar had solidified, while the bottles lay flat, two 4-mm. discs of agar bearing the fungus were planted in suitable position in each bottle. This inoculum was secured by pouring a spore suspension onto potato-dextrose agar and incubating until a mat was formed. A stiff wire loop was used to cut out the pieces. In each of the 2 series, 5 bottles of each isolate were incubated at each temperature and records taken weekly. The data for each isolate are averaged for the 2 tests and shown in figure 1.

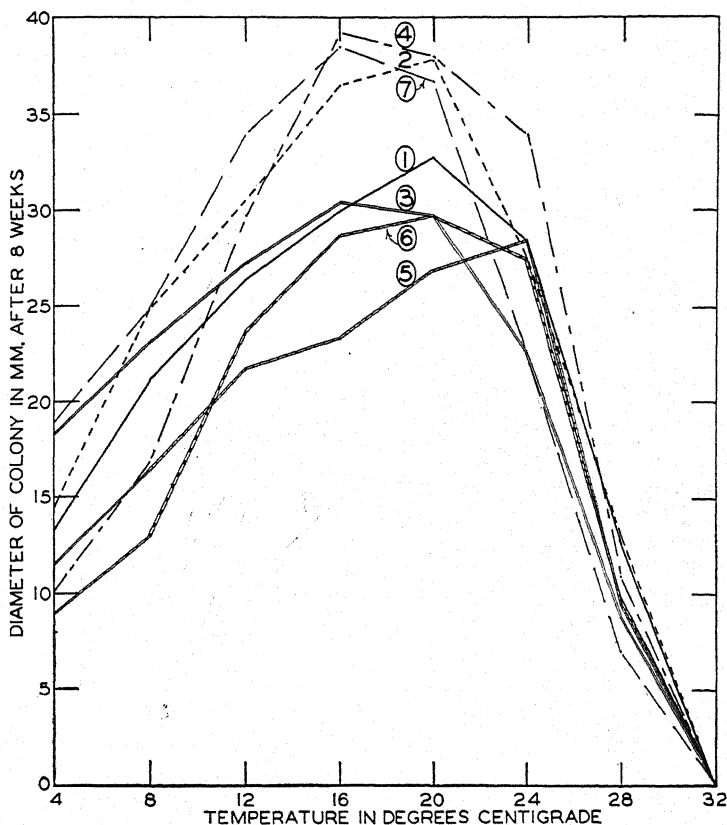


FIG. 1. Effect of temperature on the growth of 7 isolates of *Pseudopeziza ribis* on potato-dextrose agar.

Hydrogen-ion Relations. The effect of hydrogen-ion concentration on the germination of conidia was determined by a series of 4 trials. The medium was prepared as follows: 4 gr. KH_2PO_4 , 0.5 gr. MgSO_4 , 17 gr. agar and 1000 cc. distilled water were mixed and sterilized in 100 cc. amounts. On the basis of extensive preliminary trials certain amounts of sterilized normal NaOH and HCl solutions were added aseptically to each 100 cc. and, after mixing thoroughly, the medium was poured into Petri dishes. Tests on pH were made of representative plates of agar, using the quinhydrone apparatus. The readings of pH were quite consistent in the different series and are averaged for the respective values. Spore suspensions were placed in drops on the agar plates and then incubated at 16° for 72 hours, when the readings were taken. The results are averaged and shown in figure 2. Further comparisons were made, although not presented here, recording the approximate germ-tube length. The curves representing these data closely

approximated those showing germination (Fig. 2), as far as optimum and range were concerned. There was, however, only slight development at pH 8.42, whereas the spores germinated fairly well. Isolate 7 made the best growth and isolate 3 the poorest.

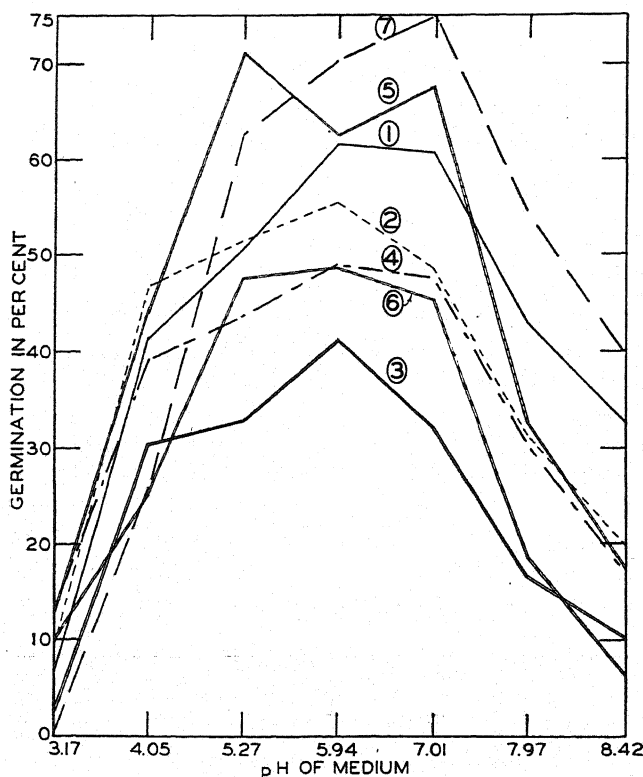


FIG. 2. Effect of hydrogen-ion concentration on the germination of conidia of 7 isolates of *Pseudopeziza ribis* on a special medium after 72 hours at 16°.

Growth of the fungus on a medium in which the pH varied was determined in 3 sets of experiments in 1933. The medium consisted of potato-dextrose agar to which were added, 4 gr. KH_2PO_4 , 0.5 gr. MgSO_4 and 10 gr. peptone per liter. Fifty cc. portions of the medium were sterilized in 12-oz. cotton-stoppered bottles. Just before the agar was ready to set, certain amounts of sterilized normal NaOH and HCl solutions were added aseptically. Some bottles were left without adding acid or alkali (pH 5.4). After being thoroughly mixed, the medium was cooled and set with the bottle lying flat. Using similar fungus mats as for the temperature series, 2 discs, 4 mm. in size, were placed in suitable position on the agar surface and the bottles incubated at 20°–22°. Readings were taken each week. One

bottle at each pH was used for each strain in the first test and 2 bottles each in the other 2 tests. All the results are averaged and shown in figure 3. This medium proved to be very well adapted to growth of the fungus. Changes in pH, which were determined for the media after the test, show the following general results. At pH 3 only very slight change in the medium, in proportion to the amount of fungal growth, occurred for the isolates tested (2-5, inclusive). At pH of 4, 5, 5.4, 6, 7, and 8 the medium changed toward approximately pH 7.5, the amount of change being directly correlated with the extent of fungus growth. At approximately pH 9, no growth of the fungus occurred, while the medium adjusted itself to about pH 8.6.

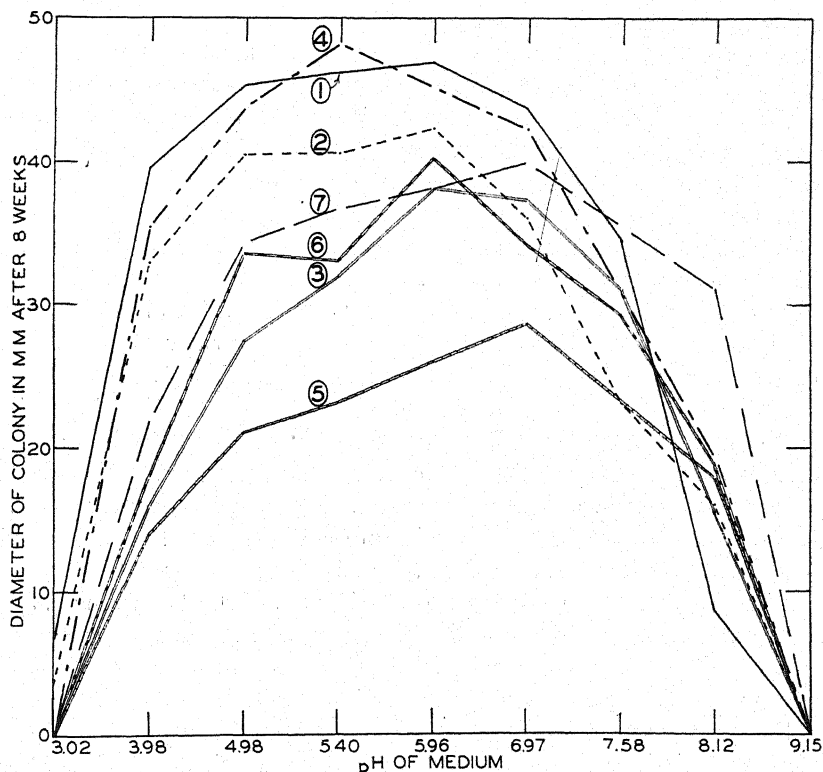


FIG. 3. Effect of hydrogen-ion concentration on the growth of 7 isolates of *Pseudopeziza ribis* on a special medium at 20-22°.

The hydrogen-ion concentration of *Ribes* leaves of several varieties kept under different temperature conditions, was tested with the quinhydrone apparatus. The results showed readings of pH 4.9 to 6.6 for gooseberry and of 5.8 to 7.1 for currant. This range includes values for samples of old and of young leaves taken separately for plants kept at about 28, 19, and 12°.

There appeared to be no consistent correlation of pH with temperature conditions of the host over a period of 2 months (determinations were made February 20, March 20, and April 17).

INFECTION STUDIES

Previous infection studies with *Pseudopeziza ribis* have been very limited. Ewert (11) by spray treatments at short intervals determined in general the time of primary infection in the spring. He also produced the disease by artificial inoculation with conidia. Klebahn (18), while demonstrating the relation of *Gloeosporium ribis* to the perfect stage, secured infection with conidia and ascospores. He was concerned primarily in later tests (19) with cross inoculations, using conidia on various species to demonstrate specialization of the fungus. The studies conducted by the writer indicate that the condition of the host plays so important a part in successful inoculations that conclusions concerning variability of the fungus, or susceptibility of the different species or varieties of the host, have sometimes been in error. At other times, correct, but apparently conflicting, conclusions have been drawn.

Lists of currant and gooseberry varieties with regard to their susceptibility have frequently been published that show conclusively that no definite statements can be made at present on the comparative or relative resistance of varieties. The Wilder has been reported by Stewart and Eustace (29) as very resistant and by Clinton as very susceptible (5). Similar cases are noted with other varieties, but, because of the confusion in varietal names of currants, some such reports are not authentic. The Long Bunch Holland variety (Thayer (32)) which probably includes the Franco-German, the Red Holland and the Red Dutch, illustrates this condition. The Franco-German is one of the most resistant sorts known, but may be heavily infected during late September, October, and November. In Germany (10) the Red Holland is reported as the most resistant variety, but it, too, may be infected late in the season. The Red Dutch is classed as very susceptible by Dutton (9). Briton-Jones (2), in 1925, made some very interesting comments concerning varietal susceptibility. He states that, at first, it would seem that the Baldwin bushes are more susceptible in a genetical sense than other varieties. Such is not the case, since heavily pruned Baldwins were no more affected than heavily pruned bushes of other sorts. The reason why it is more often and more severely affected is due to the fact that it exhausts itself by heavy cropping and is a weak grower. "The hereditary factor, therefore, is not one of susceptibility to disease but one of heavy cropping and weak growth. Without a critical study of the conditions of plants it is unsafe to state that any one variety is more susceptible to disease than another." From such reports in literature and from studies and

observations by the writer, it is concluded that all commonly cultivated varieties of red, white, and black currants, and gooseberries may be infected by *Pseudopeziza ribis*, their comparative susceptibility being influenced largely by environmental conditions.

The infection studies reported in this paper were made on No. 1, 2-year-old currant and gooseberry plants bought from the McKay Nursery Co., Madison, Wisconsin. The bushes were planted in good soil in 8-inch pots and most were kept at about 19°. They were watered sufficiently to prevent severe wilting. By experience it was found that moderate to poor growth of the host was more favorable for infection by the fungus.

In all, over 400 plants were inoculated, using the following varieties: currant, Wilder, Perfection, Cherry, Diploma, White Grape, Fay's Prolific and Red Cross; gooseberry, Red Jacket, Downing and Pearl. All 11 isolates were pathogenic, although in varying degrees, and all varieties tested were susceptible. Conidia of cultures isolated from currant when inoculated on currant gave better infection than on gooseberry. Likewise, isolates from gooseberry were more pathogenic on gooseberry. The plants were inoculated by atomizing the lower, or upper, or both leaf surfaces with a suspension of conidia in distilled water, and also by discharging ascospores onto the leaf surface. The bushes were then placed for definite periods in a moist chamber described by Keitt and Jones (16). Results were taken after 2 weeks or longer.

TABLE 5.—*Relation of temperature of Ribes plants before inoculation to infection of excised leaves by conidia of Pseudopeziza ribis*

Host plants ^a	Temperature before inoculation, ^b ° C.	Results of inoculation of excised leaves on stated dates and leaf surfaces: ^d			
		March 21 ^c		April 19	
		Upper	Lower	Upper	Lower
Currant	28	1.52	17.04	2.61	6.45
	12	.75	2.90	1.28	.52
Gooseberry	28	3.12	24.57	80.20	95.00
	19	2.71	12.21	42.70	97.60
	12	.93	6.32	2.87	57.40

^a These plants are the same used in the experiment on pH of *Ribes* leaves.

^b All plants had been growing at about 19° for 30 days when they were placed at these temperatures on February 20.

^c Excised leaves 19–32 in number were incubated in moist dishes at 20° for two weeks after inoculation with conidia; isolate 5 on currant, isolate 2 on gooseberry.

The inoculations of excised leaves in moist dishes proved to be as reliable as those on potted plants. It should be pointed out that the behavior of the fungus was as erratic in these studies as it was in spore-germination trials, although no correlation could be drawn between successful inoculations and germination of the spores on agar plates. The data given, while representing rather limited numbers of plants, are considered a fairly accurate representation of the results of the 3 years.

Factors influencing infection. The effect of different temperatures on susceptibility of the plants is shown by the experiments on excised leaves reported in table 5 and by more extensive trials on entire potted plants. With the exception of a single trial on Downing, in tests not reported, the plants held at higher temperatures were much more susceptible. Since records were taken on both old and young leaves, these differences cannot be attributed to the effect of temperature on extent of growth, favored by lower temperatures, but rather to the condition of the plant, particularly the leaf. That morphological changes account for the differences seems unlikely.

In a test on April 25, 1932, leaves of Wilder and an unknown currant were inoculated with spores of isolate 5 after injuring the upper leaf surface. After 2 weeks the numbers of lesions per square inch were as follows:

	Lower surface	Upper surface	Upper surface brushed with a stiff fiber brush
Wilder	11	7	75
Unknown	159	5	200

Other tests and observations have shown that injuries to leaves, or other unfavorable conditions, increase their susceptibility.

The data given in table 6 show conclusively that infection occurs mainly through the lower leaf surface. There are a few exceptions, however, scattered throughout the inoculation experiments. Studies with ascospores show also that penetration is more common on the lower surface.

The length of moist period after inoculation necessary for the fungus to establish itself sufficiently to induce infection is shown to be much shorter than the time curve of germinating spores would suggest. The experiments reported in table 7 permit one to conclude in general that a moist period of 12 to 24 hours is sufficient for infection by conidia. No study has been made of the moist period necessary for infection by ascospores. Extensive data on the relation of temperature during the moist period to infection show that infection may occur at 10° to 28° but none at 32°. In some cases no disease appeared on plants inoculated at 28°. Tests with excised leaves held at 8° gave good infection, but lesions were very slow to appear. The optimum

TABLE 6.—*Comparative susceptibility of upper and lower surfaces of currant and gooseberry leaves to infection by conidia of Pseudopeziza ribis*^a

Date	Variety	No. of bushes	Surface inoculated	Av. No. lesions per $\frac{1}{4}$ sq. in. per stated leaf: ^b		
				1	2	3
March 8, 1933	Red Jacket	1	lower	50.0	53.7	30.3
do.	do.	1	upper	4.7	10.0	3.9
March 18	Downing	2	lower	33.9	28.0	13.3
do.	do.	1	upper	16.1	14.0	9.5
March 20	do.	2	lower	11.3	4.7	2.0
do.	do.	do.	upper	1.7	.7	0
March 23	Red Jacket	do.	lower	34.3	31.0	14.1
do.	do.	do.	upper	6.0	3.7	3.1
April 30	Currant	4	lower	37.9	38.6	46.4
do.	do.	3	upper	1.6	.6	.5

^a The gooseberries were inoculated with spores of isolate 2 from bean pods, the currants with spores of isolate 2 from leaves and of isolate 5 from potato-dextrose agar. The plants remained for 48–86 hours in a moist chamber at 16–20°.

^b Data were taken on 25 gooseberry and 4 currant shoots per plant, choosing those most heavily infected and counting the lesions on 3 leaves per shoot. These represented the most severely infected leaves on (1) the lower, (2) middle, and (3) upper third of each shoot.

temperature for infection by conidia apparently is from 16° to 24°, as indicated by tests both on potted plants and excised leaves. Ascospores were discharged onto plants held at 12° and at 20° and good infection developed (Plate I, B, F).

Several infection studies were conducted on potted plants and excised leaves using ascospores from currant and gooseberry leaves in 1933 and 1934. In some tests, pieces of leaf bearing ascocarps were moistened on a cover slip placed on a rubber washer. The cell thus made was then clamped on either leaf surface with the ascocarps oriented towards the leaf, and the plant placed in the moist chamber. This method, which was described by Keitt (15), is very useful and the results are striking. Lesions appear in a circular area the size of the aperture of the rubber washer (Plate I, C).

The usual method of inoculating plants with ascospores was to lay the potted plants in the moist chamber at a suitable angle, with the branches so arranged that the lower leaf surface was uppermost and held in place by a tray of large-mesh wire netting. Petri dishes with moist filter paper in the bottom on which adhered Ribes leaves with abundant ascocarps were inverted

TABLE 7.—*Relation of the length of moist period after inoculation with conidia to infection by Pseudopeziza ribis*^a

Date	Variety	Temperature before inoculation ^b ° C.	No. hours in moist chamber	Av. No. lesions per $\frac{1}{4}$ sq. in. per stated leaf: ^c		
				1	2	3
April 27, 1933	Diploma	19	12	5.0	6.2	4.2
do.	do.	do.	18	5.0	10.2	12.0
do.	do.	do.	24	6.7	11.6	16.7
do.	do.	do.	48	4.3	4.0	12.9
March 25	Cherry	28	24	2.4	2.0	1.8
do.	do.	do.	48	6.9	9.9	5.7
do.	do.	do.	72	2.7	2.8	1.8
do.	do.	do.	96	2.8	2.9	2.4
April 5	Red Jacket	19	24	33.0	22.2	18.9
do.	do.	do.	48	40.8	36.5	35.0
do.	Downing	28	24	47.1	23.8	9.0
do.	do.	do.	48	95.5	83.4	59.0
do.	do.	do.	72	82.1	84.2	60.2
March 25	do.	do.	48	47.8	46.3	22.1
do.	do.	do.	72	35.1	21.9	11.2
do.	Red Jacket	do.	48	45.2	30.9	25.1
do.	do.	do.	72	61.1	47.5	37.3

^a In the March 25 test, conidia of isolate 6 from potato-dextrose agar were used on currant; in all others spores of isolate 2 from leaves. After inoculation of the lower leaf surface, the plants were held in the moist chamber at 16 or 20°.

^b The plants were grown at this temperature since February 20.

^c Data were taken on the most heavily infected leaf on (1) the lower, (2) middle, and (3) upper third of each shoot. One plant was used in each case, and the data were averaged for 25 gooseberry and for 3–16 currant shoots.

on this wire tray. The plants were sprayed with distilled water and kept in the chamber as long as desired, 48–96 hours. This method of inoculating with naturally discharged ascospores proved very satisfactory, and severe infections were secured (Plate I, B, F). Cross-inoculations, using ascospores from currant, showed definitely that the fungus was more pathogenic on currant than on gooseberry. Similarly, ascospores from gooseberry caused more infection on leaves of gooseberry than on leaves of currant. Because of variations in inoculum, quantitative readings were not attempted. The conidia produced as a result of these inoculations were extremely pathogenic in comparison with spores from artificial cultures. The varieties used were Diploma, Perfection, and Red Jacket, all with growing shoots. Ascospores as well as conidia gave better infection on the older leaves.

To determine whether ascospores could infect very young leaves, however, shoots of Diploma and Poorman were gathered in the garden on April 25, 1933, and, after tying the cut ends in cheesecloth and absorbent cotton, small bunches were fitted like corks in the tops of pint milk bottles containing water. These were partly inverted in a moist chamber and the shoots inoculated as usual with ascospores from currant. When picked, the buds were just beginning to break, and during the moist period they continued to open. Good infection appeared on the small leaves after about 9 days.

Pathological histology. No studies of the manner in which the fungus gains entrance to the host have been reported. Ewert (13), in testing control measures, found that spraying either of the leaf surfaces alone with Bordeaux mixture did not prevent infection, while no disease developed if both surfaces were covered with spray. It would thus appear that infection may take place on both leaf surfaces. Several experiments by the writer with both ascospores and conidia have shown that such is the case. As mentioned before, the uncertain manner in which spores may germinate and infect the host has presented even greater difficulties in penetration studies than in other phases. Many inoculation tests were performed on potted plants held in a moist chamber and on excised leaves in moist dishes, using a wide range of conditions and of methods for applying spores to the leaves. Pieces of tissue were fixed, imbedded, sectioned, and stained. Many variations were tried in fixatives and stains. In all the currant leaves used, certain cells, especially in older tissue, contain materials that stain very heavily and interfere seriously with this operation and with examination. The best results were secured on Downing leaves previously infected and bearing a few lesions. These leaves were picked, washed in a small amount of water, thus spreading the spores from the acervuli, and placed in moist dishes. The wash water was then atomized onto both leaf surfaces and the leaves were incubated at 20°. Fixations were made in Gilson's fluid after 48, 96, and 144 hours. After embedding, the material was sectioned 7-8 μ thick and stained with safranin and fast green. Certain stages of penetration and development of the fungus in the leaf were drawn with the aid of a camera lucida and are shown in figure 4, A-D.

Observations also were made on material treated as follows: Leaves were inoculated by atomizing a spore suspension on one or both surfaces and incubating in moist dishes at certain temperatures. After certain periods of from 24 to 96 hours, pieces of leaf were cut out and the chlorophyll was removed in a solution containing equal parts of absolute alcohol and glacial acetic acid. Then the material was cleared in a saturated solution of chloral hydrate. The tissue was placed on a glass slide in lacto-phenol, heated slightly, and then stained by warming with acid fuchsin dissolved in lacto-phenol. These made very satisfactory temporary mounts for observing

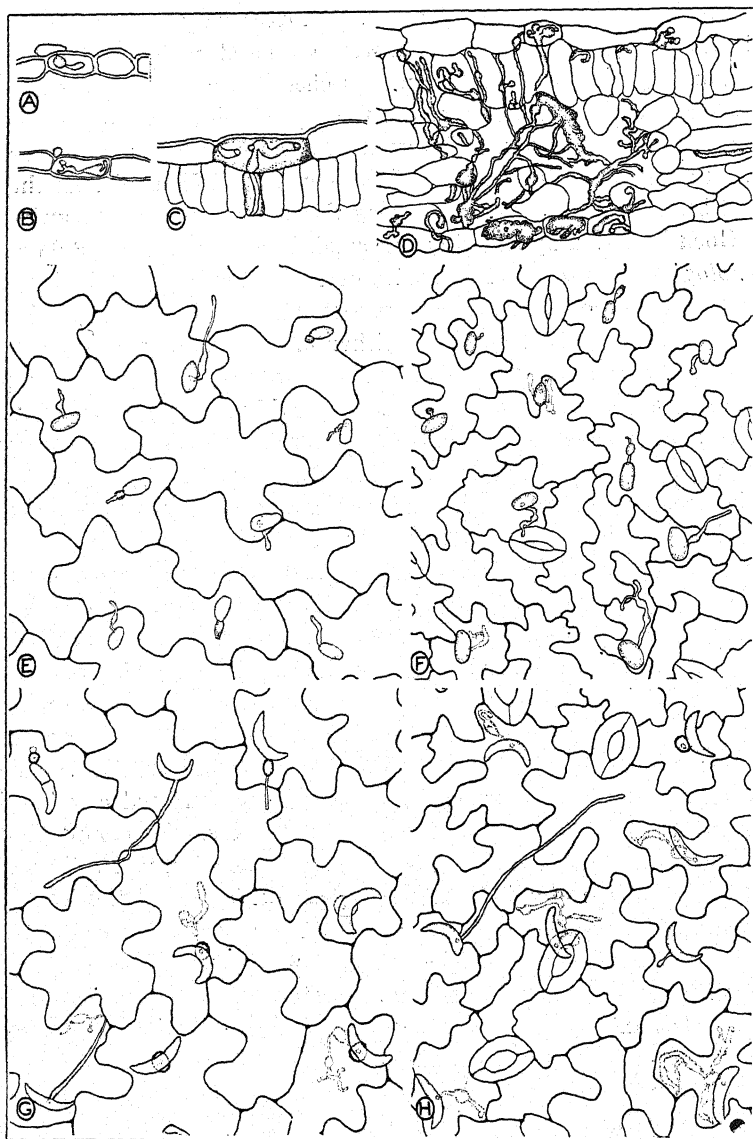


FIG. 4. Penetration and development of *Pseudopeziza ribis* in leaf tissue. Drawings made with the aid of a camera lucida. $\times 250$. A. Early stage of penetration of Downing leaf following germination of conidium. B. Later stage, showing enlarged primary hypha. C. Further development into the palisade layer with killing of the cells. D. Lesion when first visible macroscopically, about 144 hours after inoculation, showing extensive fungus development. E. and F. Ascospores on upper and lower surfaces, respectively, of *Diploma* leaf. G. and H. Conidia on upper and lower surfaces, respectively, of Downing leaf. Dotted lines represent the fungus inside the leaf.

spore germination and penetration by the fungus. Such studies were made, using conidia on both surfaces of Downing leaves and ascospores on both surfaces of Diploma leaves. Illustrations showing various types of germination and several stages of penetration are given in figure 4, E-H. The mode of penetration in conidial infection by *Pseudopeziza ribis* is shown to be directly through the cuticle and epidermal cell wall.

Conidia that germinate on a leaf surface may or may not produce an appressorium. On the lower surface in many cases the conidium itself acts as an appressorium and the infection hypha penetrates directly beneath the spore. From the spore or from the appressorium, as shown by a germ pore, a small projection or infection hypha is forced down through the cuticle and the epidermal cell wall into the epidermal cell (Fig. 4, H). Here the fungus forms a primary hypha that varies widely in size and shape. Most commonly it is a rather large globular body with one or more branches. These are fine or very large and of irregular shape, generally confined to the same or closely adjacent epidermal cells. This secondary growth may completely fill an epidermal cell (Fig. 4, D) before or simultaneously with the development of slender hyphae that extend into the intercellular spaces and palisade cells, invading all the tissues including the vessels. Large irregular, fungal bodies may be scattered throughout the infected tissue. At about the time the lesions appear the mycelium tends to accumulate just below the epidermal cells, and there fruiting takes place, pushing the epidermis up, rupturing it, and liberating the spores. Parasitized cells die within a short time and may become completely disintegrated, leaving large cavities or spaces filled with fungal growth.

What takes place in the host, if penetration occurs, when a resistant leaf is attacked has not been determined. It is known, however, that spores may germinate readily on leaves that never show lesions. It is possible that in these cases the spores send out long, slender germ tubes that generally stay on the surface. No cases of stomatal penetration were observed. The very simple preparation for penetration, together with the extensive development of the fungus just after entrance, may play an important part in the ready establishment of the fungus. The facts, too, that the cuticle and upper epidermal walls are thicker than those of the lower epidermis may explain in part the data shown in table 6. It should be noted, too, that bushes in proper nutrition possess a thicker cuticle and also more resistance to the disease. Plants grown in the sun, however, are considered to form heavy cuticular layers, but they are also more susceptible than bushes grown in the shade, according to Stewart and Eustace (29).

Studies of penetration by ascospores show essentially the same type as described for conidia (Fig. 4, E, F). Very extensive observation on material from several inoculations, however, showed few examples of penetration;

in fact, on the upper surface very few ascospores were seen germinating and no conclusive evidence of penetration was found. A fair percentage of ascospores germinated on the lower surface, but the fungus was extremely difficult to see after penetration. The writer is of the opinion that the drastic treatment in preparing the leaves literally disrupted the fungal body, leaving granular contents with no definite outline beneath the germinating ascospore.

LIFE HISTORY STUDIES

Especially in regions with severe climate, the mode of overwintering of parasitic fungi is of very great importance. Dudley (8), as early as 1889, in what was then the most complete work on currant anthracnose, stated that . . . "the biological investigation of this (the fungus) presents peculiar difficulties for we have little doubt that it hibernates elsewhere than on the fallen leaves of the previous season." Stewart and Eustace (29) twelve years later first reported the occurrence of the fungus on young canes. Not until 1906, however, when Klebahn, by satisfactory inoculation and pure culture tests, proved the relation of *Gloeosporium ribis* to a Discomycete, was the most important method of overwintering actually demonstrated. That this stage is extremely rare or is very difficult to find is evident from the few records in literature, and so far as the writer is aware his specimens of the perfect stage are the only ones that have been collected in this country.

Several careful examinations were made of leaf remnants in the *Ribes* plantation at Madison in the spring of 1931. No ascocarps could be found. No better success was had in observations at Sturgeon Bay the same spring. That fall, in Madison, large quantities of heavily infected currant leaves were picked and placed in cloth netting bags on sod for overwintering. By February only a few pieces of petioles remained. Throughout the winter, leaves had been gathered in the currant planting and a series of fixations made. By the middle of April, 1932, the leaves had so disintegrated and disappeared that no more material was available.

The first *Pseudopeziza ribis* fruiting bodies seen by the writer were found June 12, 1932, on overwintered leaves of wild black currant (*Ribes americanum*) at Sturgeon Bay, Wisconsin. Only a few ascocarps were found and no studies other than fixations were possible. On cultivated red-currant leaves mature ascospores were found on material collected May 12. Fruiting bodies were very rare in this material, which had been overwintered in cloth-netting bags on sod.

Again in October, 1932, infected leaves were placed in cloth-netting bags and in open wire pens for overwintering studies at Madison. On February 9, 1933, some leaves were collected and placed in moist dishes and incubated at 8° and 12°. They were not observed until March 16, when abundant ma-

ture ascocarps were found. Spores were discharged on potato-dextrose agar for isolation and pieces of leaf tissue were fixed. Numerous other collections were made at various intervals until May 1, forcing out the ascocarps for use in this study. On April 10, 1933, some gooseberry leaves that had been collected in the garden produced mature ascocarps after having been held for 2 weeks at 12°.

After returning to Sturgeon Bay in May, 1933, the writer resumed observations on the same Ribes plants as before. No perfect stage was found on the wild black currant in 1933. Although most of the cultivated currant leaves were decayed, there were abundant ascocarps on the leaf fragments. It was surprising, indeed, to find the gooseberry leaves well preserved and with abundant apothecia (Plate II, O). Large collections were made, isolations secured and material fixed. The ascospore-discharge studies, shown in figures 5 and 6, were made with leaves of this material. A small package of leaves was gathered May 15, stored out of doors, and, on May 27, mailed to Madison to be placed at 4°. On February 7, 1934, some of these leaves were moistened and placed over currant and gooseberry bushes in a moist chamber, and abundant infection resulted from the ascospores discharged. On April 17, these ascospores were still highly viable. At the last test, December 2, 1934, the spores discharged readily but very few germinated.

In the fall of 1933 diseased currant leaves of the Franco-German variety, considered highly resistant to this fungus, were collected and laid down as usual. Throughout this investigation leaves of this variety have proved to be the most favorable for overwintering studies. Collections of this material were made at intervals beginning in December and placed in moist dishes at 8° and 12°. The leaves collected January 13, showed ascocarps that contained mature spores after 30 days at 8°. The best temperature for maturing the perfect stage depends on the time of collection or stage of fungus development. For early material in January and February, 8° is best and later 12–16–20° hasten ascocarp maturation. This organism appears to have a graduated thermal requirement for ascocarp development somewhat similar to that described by Wilson (33) for *Venturia inaequalis*. If the leaves are kept at 22° or above, few ascospores are formed but, rather, conidia or sterile ascocarps. That these semi-natural conditions are favorable for ascocarp development is shown in plate II, P.

During the winter of 1933–34 fixations were made of heavily infected Franco-German leaves wintering under natural conditions. Collections were made about the middle of each month beginning in December. All material was fixed in F. A. A., inbedded in paraffin, sectioned 8–10 μ thick and stained with safranin and fast green. For comparison, the series prepared in 1931–32 was reexamined and found to agree with the one of 1933–34. To show certain stages in the development of the ascocarps on Franco-German leaves photomicrographs are presented in plate III, A–H.

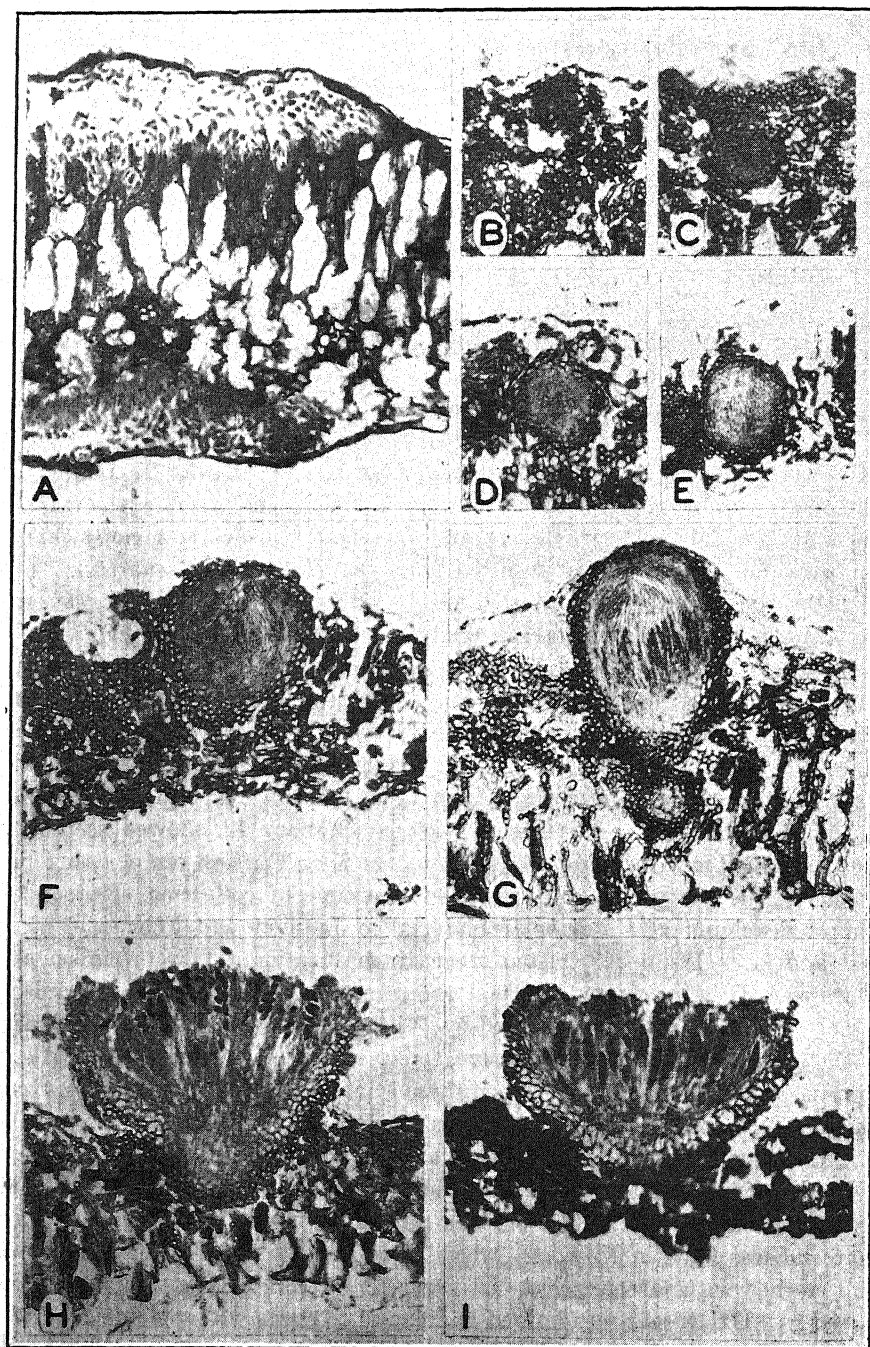


PLATE III

At the close of the vegetative period of *Ribes* plants the fungus in diseased leaves produces large numbers of conidia and microconidia. These may be borne together or in separate acervuli (Janeczewski and Namyslowski (14)). The mycelium is mainly hyaline and fine and is largely restricted to the lesion. Later in the season, especially after the leaves have fallen, the fungus apparently enters a saprophytic stage and the mycelium extends in the tissues and enlarges, its walls become thick, and heavy mats of pseudoparenchymatous tissue are formed beneath the acervuli (Plate III, B). The initials of the fruiting body may arise in this mass (Plate III, C), or elsewhere along the extended mycelium as small groups of twisted hyphae. Unfortunately, the morphological and cytological details of ascocarp development are not known; therefore, it remains to be determined whether or not there are sexual processes involved. The ascocarp initials are formed at varying depths in the tissue of the leaf, and as they enlarge and mature they approach the surface, finally emerging in a typical, flat-top, disc-shape apothecium, sessile or slightly stalked. Normally the fruiting bodies will mature only on the lower leaf surface, although occasionally they occur on the upper.

Small portions of blades and of petioles or entire leaves protected in the crown of the bushes may provide ascospore inoculum for primary infection. In nature, ascospores were mature on Franco-German leaves at Madison about April 27, 1933, and at Sturgeon Bay on cultivated gooseberry leaves about May 12. The records thus show that mature spores are present about the time the bushes foliate. That these very young leaves are susceptible was shown by an experiment described earlier in this paper.

Primary infection in the spring may be initiated by ascospores, by conidia formed in the spring, and probably by conidia formed in the fall. The production of ascospores has been described. These spores are discharged only when the leaves are moistened with rain or dew and may be carried by air movements to susceptible parts to which they adhere very tenaciously. At temperatures of 16–22° in the greenhouse infection appears in 8–9 days after inoculation with ascospores.

PLATE III. Stained sections of Franco-German leaves, showing certain stages in the development of the ascocarps of *Pseudopeziza ribis*. $\times 165$. A. Collected just previous to leaf fall, October 11, 1932, showing acervuli with spores on both leaf surfaces. Microconidia are present but do not show here. B. Collected December 13, 1933, showing extensive development of the heavy-walled mycelium and an apothecial initial. C. Collected January 13, 1934. D. Collected February 12, 1934. E. Collected March 12, 1934, showing the beginning of differentiation of the contents of the fruiting body. F. Collected April 12, 1933, showing a later stage. G. Collected April 12, 1933, showing the young asci. H. Mature ascocarp with spores. There is some indication of impoverishment of the mycelium in the host. I. Mature ascocarp on gooseberry leaf.

Conidia may be formed in abundance on the overwintered leaves. Klebahn (20) assumed that the fungus on gooseberry overwintered in this manner instead of by ascospores. The writer has found conidia being produced on overwintered gooseberry and currant leaves either in stromatic acervuli or more or less definite pycnidium-like bodies (Plate II, M). Microconidia have also been observed in abundance on overwintered leaves, but their exact origin whether in acervuli, ascocarps, or pycnidia, was not determined. That conidia produced on overwintered leaves are able to infect green leaves, was shown in a test on April 12, 1933, whereby excised Downing and Red Jacket leaves were inoculated in a Petri dish at 22° with spores from overwintered gooseberry leaves that had been kept at 12° for several days. This experiment gave abundant infection within 7 to 8 days. It is highly probable that these conidia may prove a source of primary inoculum.

That conidia of *Pseudopeziza ribis* would remain viable over winter, was first reported by Ewert (12). He did not secure infection with them, however. The writer has on several occasions secured germination of conidia from overwintered leaves taken directly from the field, but has no proof that they will infect leaves. Abundant spores on leaves in midwinter after warm periods indicate that spores may be formed at any time the temperature is above freezing. Thus spores found on overwintered leaves may have been formed only a short time previously.

During the summers of 1931 and 1932 at Sturgeon Bay, the writer made frequent examination of a currant planting and kept general records of disease and host development. These records agree in general with the data presented in detail for 1933 and 1934. The particular planting observed was composed of 39, 20-year-old gooseberry, 35 Cherry currants and 2 white currant bushes, all poorly cared for by the owner. It was approximately 3 miles from the station where weather data were recorded. Notes on host development and progress of the disease, including defoliation and leaf spotting, were followed on 10-20 shoots of 2 nonsprayed bushes of each variety. The ascospore discharge record was taken on 5 gooseberry leaves by the method used by Keitt and Jones (16). It consisted essentially of placing a small glass slide smeared with glycerine jelly about $\frac{1}{8}$ inch above each leaf, which rested flat on the ground. Each day at 8 A. M. the slides were changed and the spores counted. The temperature, rainfall and humidity records were taken from continuously recording instruments. Special attention is invited to the data relating to epidemiology presented in figures 5 and 6. The comparatively early and severe outbreak of the disease in 1933 may be correlated with the abundance of early primary inoculum and the occurrence of favorable, moist weather conditions. In 1934, after a slow start, the disease became extremely severe and spread rapidly late in the season.

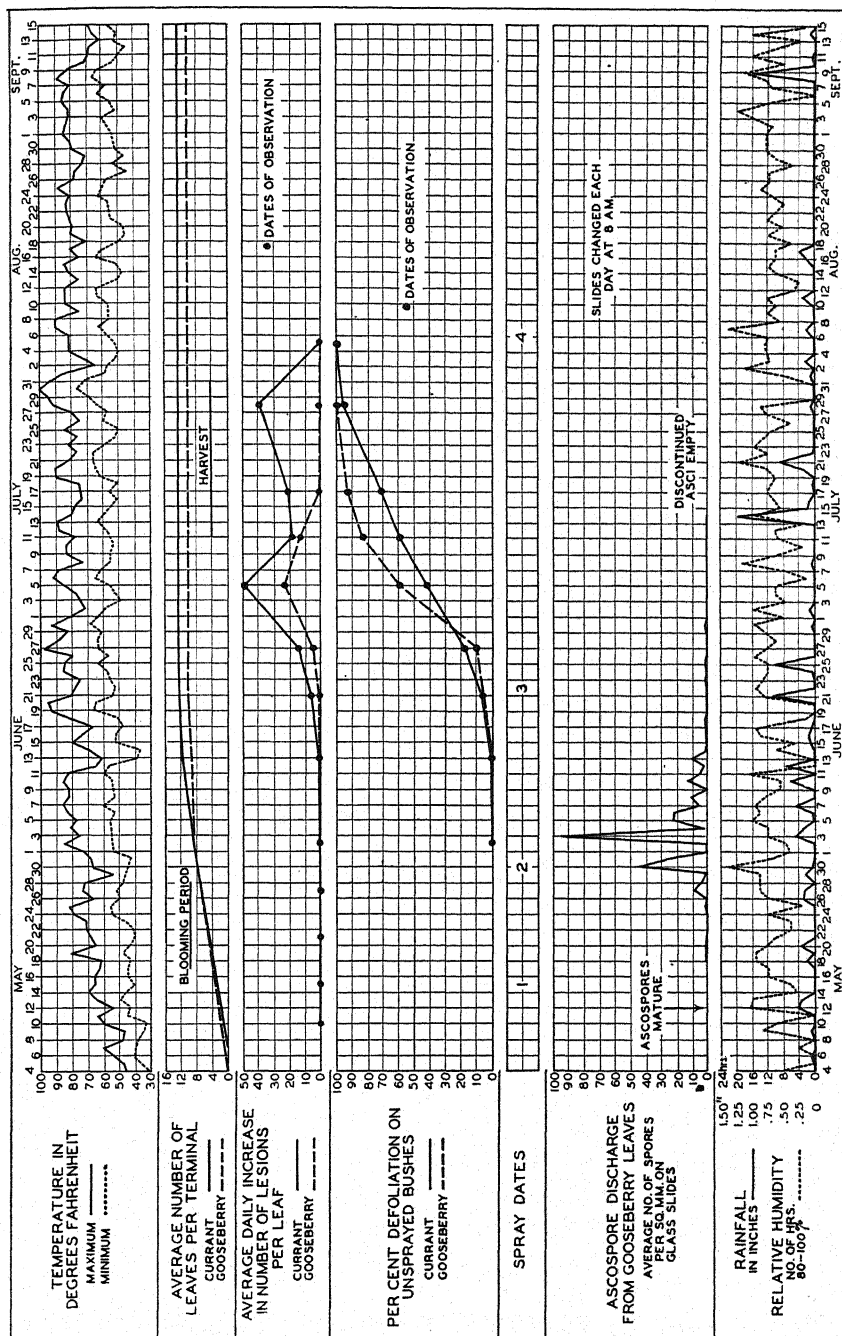


FIG. 5. Graphic representation of several factors important in the epidemiology and control of currant and gooseberry anthracnose, Sturgeon Bay, Wis., 1933. (See table 8 for results of spray treatments and text for further explanation.)

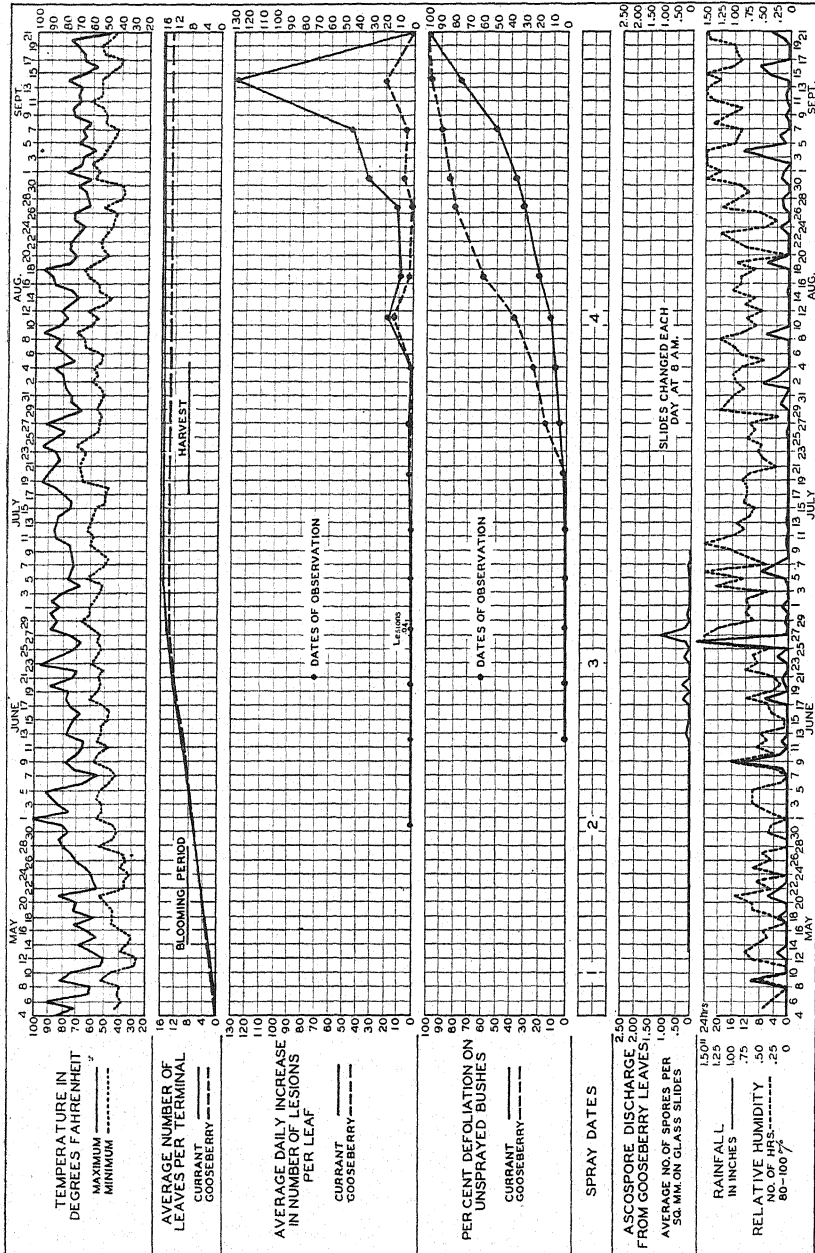


Fig. 6. Graphic representation of several factors important in the epidemiology and control of currant and gooseberry anthracnose, Sturgeon Bay, Wisc., 1934.

CONTROL

Contributions to the development of satisfactory control measures for anthracnose have been made by many workers over a wide range of conditions. Dudley (8), in 1889, suggested the use of "one of the copper solutions" if a severe outbreak seemed evident. The first experiments of consequence apparently were those by Ewert (10), in 1905. He reported the results of an extensive experiment using various copper sprays all of which gave good control. He also found that heavily pruned bushes were much less severely affected than his checks.

Ewert confirmed his conclusions regarding the effectiveness of copper sprays in very extensive experiments reported in 1907 (11) and further trials in 1913 (13). Noffray (28) in France recommended cutting out the disease as it appeared. The experiments in England by Marsh and Maynard (24, 25, 26) on black currants mainly of the Baldwin variety show conclusively that Bordeaux mixture is very effective in controlling anthracnose. Further striking proof is given of the value of maintaining vigorously growing plants through adequate nutrition and by pruning methods.

In the United States, Stewart (30, 31), working with nursery stock, found lime-sulphur and Bordeaux mixture to be effective if applied at the proper time. Dutton (9), after extensive trials, concluded that Bordeaux mixture, lime sulphur, and dusting sulphur were satisfactory, but the former was more efficient. Caesar (3), in Canada, reported good control with Bordeaux mixture, less satisfactory control with lime-sulphur and poor results with soluble sulphur and copper-lime dust.

Since the discovery by Klebahn (18) of the fruiting bodies on overwintered leaves, destruction of the old foliage is generally recommended.

Spraying experiments for the control of anthracnose were conducted by the writer in 1932, 1933, and 1934 on the planting at Sturgeon Bay in conjunction with life-history studies reported earlier in the paper. In 1932 the experiment was on a small scale, involving the use of a knapsack sprayer. Very good results were secured in a program similar to that of 1933 and 1934 with the Bordeaux mixture and lime sulphur.

The treatments in 1933 and 1934 were applied with a barrel pump to the whole planting, except 2 currant and 2 gooseberry bushes for checks. Generally 5 or more bushes of each variety were sprayed when the full spray schedule was followed, while only 1 or 2 bushes were treated with each of the reduced programs. The materials used, dates of spray application and comparative results of the treatments in 1933 are shown in table 8. In this table also are given the data on control of the gooseberry powdery mildew (*Sphaerotheca mors-uvae* (Schw.) B. and C.). In these tests and in 1934, lime sulphur provided complete protection from mildew, while Bordeaux mixture gave quite poor control of this disease. Where the two diseases are

TABLE 8.—*Relation of spray treatments to control of currant anthracnose and gooseberry powdery mildew, Sturgeon Bay, Wisconsin, 1933*

Spray treatment ^a	Fruits mildewed on June 27 ^b	Approximate defoliation on Aug. 5	Defoliation on Sept. 14
	%	%	%
Currant			
B. M., 1, 2, 3, 4	0	23.1
do. 2, 3, 4	5	32.1
do. 1, 2, 3	0	38.9
do. 2, 4	55	97.1
Nonsprayed	100	100.0
L-S., 1, 2, 3, 4	5	31.4
do. 2, 3, 4	10	50.8
do. 1, 2, 3	5	85.9
do. 1, 2, 4	10	58.5
do. 2, 4	90	100.0
Gooseberry			
B. M., 1, 2, 3, 4	61	0	28.7
do. 2, 3, 4	67	20	39.8
do. 1, 2, 3	61	0	52.2
do. 1, 2, 4	68	20	87.5
do. 2, 4	63	85	96.8
Nonsprayed	90	100	100.0
L-S., 1, 2, 3, 4	0	5	81.8
do. 1, 2, 3	0	5	98.0
do. 2, 3, 4	28	93	100.0
do. 2, 4	34	98	100.0

^a B. M. = Bordeaux mixture, 3-4-50, using high-calcium hydrated lime.

L-S = Liquid lime sulphur, 1-40.

Spray dates: 1, May 15; 2, May 30; 3, June 22; 4, August 6.

Arsenate of lead, 1-50, was used in sprays 1, 2 and 4.

^b Only sprays 1, 2 and 3 had been applied at this time, and probably spray 3 had not yet influenced the counts at all.

Data were taken on 100-600 fruits per treatment.

present in the same planting, and because of danger of lime-sulphur injury, a mixed spray program is recommended by Lovett and Barss (22). In 1934, at Sturgeon Bay, a similar schedule proved very satisfactory. The first two sprays of lime sulphur for gooseberry mildew were followed the rest of the season with Bordeaux for anthracnose. Convincing evidence was found that foliage of weak gooseberry bushes was severely burned by lime-sulphur sprays during hot weather, while healthy bushes close by showed no injury.

Results from the spray programs tested during the 3 years indicate that Bordeaux is more efficient than lime sulphur in controlling anthracnose. The Bordeaux treatment before bloom is the least important.

The Bordeaux program for currants and the lime-sulphur-Bordeaux schedule for gooseberries, with thorough application of spray, combined with proper cultural methods, may be expected to give satisfactory control of anthracnose and mildew under similar conditions.

SUMMARY

A study has been made of currant and gooseberry anthracnose, caused by *Pseudopeziza ribis* Kleb., considered the most wide-spread and destructive disease of cultivated *Ribes*.

Isolates of this fungus from Wisconsin, Oregon, Canada, and a culture from Europe have been studied. These showed rather wide, consistent differences in several morphological and physiological characters. All isolates studied proved to be pathogenic, but in different degrees. A method was used for measuring curved spores, and spore measurements for the several isolates are presented.

The optimal temperature for germination of conidia was about 20°, for ascospores about 12°, and for growth of the fungus on solid media about 20°. The isolates varied slightly in optimum temperatures and greatly in rate of growth. A temperature of 32° killed spores and mycelium in culture. Conidia were produced in greatest numbers at about 20–24° and microconidia most abundantly at about 8–16°. Ascospores were discharged from leaves at 1–32°.

The optimal pH for growth under the cultural conditions studied was between 5.4 and 7.0, and approximately the same for germination of conidia. Conidia were produced in greatest numbers at pH 4.0 and microconidia were produced abundantly throughout the range supporting growth.

The pH of freshly macerated currant and gooseberry leaves tested on three dates varied approximately from 5.0 to 7.0.

Infection studies were conducted on several varieties of currants and gooseberries, using conidia of all 11 isolates and ascospores from currants and gooseberries. The spores from currants were more pathogenic on currants than on gooseberries and *vice versa*. Plants inoculated on the lower leaf surface were more heavily infected than those inoculated on the upper surface. Plants held at higher temperatures prior to inoculation were more susceptible to the disease than those held at lower temperatures. Infection occurred at favorable temperatures with a moist period of 12 or more hours after inoculation, and approximately 20° was the optimal temperature for infection.

The mode of penetration, whether from conidial or ascosporic inoculation, was directly through the cuticle and the epidermal cell wall of either epidermis. Appressoria often functioned in this process. Both inter- and

intracellular development involved all leaf tissues and disorganized and killed the cells.

Development of the fungus during the fall, winter and early spring has been followed in sectioned and stained preparations and by field observations. Primary infection in the spring is initiated by ascospores, by conidia formed in the spring, and probably by conidia formed in the fall. Ascospores may infect the host as soon as the leaves unfold in the spring.

A method was developed for handling overwintering Franco-German leaves, whereby mature ascospores were available by March 1. Experimental work was greatly facilitated by the use of viable ascospores from gooseberry leaves stored at 4° from May, 1933, to April, 1934. The perfect stage on *Ribes grossularia* is reported for the first time.

A graphic summary of data on host, fungus and disease development in relation to meteorological records at Sturgeon Bay is given for 1933 and 1934.

The writer's studies and evidence recorded in the literature show that no definite statement on varietal resistance can safely be made at present. Their comparative susceptibility depends largely on environmental factors.

Experiments at Sturgeon Bay during the seasons of 1932, 1933, and 1934 indicate that Bordeaux mixture, 3-4-50, will give satisfactory control of anthracnose under the conditions encountered if thoroughly applied: (1) just before the plants bloom, (2) just after fruit set, (3) three weeks later and (4) just after harvest. Lime sulphur, 1-40, in the same program was less effective for anthracnose but highly efficacious in control of powdery mildew of gooseberry. A mixed-spray program, in which lime sulphur was applied for the first 2 sprays and Bordeaux mixture for the later ones, gave good control of both anthracnose and mildew.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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THE BIOLOGY OF GANODERMA LUCIDUM ON ARECA AND COCOANUT PALMS

S. V. VENKATARAYAN

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INTRODUCTION

The paucity of literature on tree and wood decay in India is probably due to the absence here of a serious factor like the *Hausschwamm*, or dry rot of Europe and America. Of late, however, the question of the rot of railway sleepers and wooden posts for electric transmission lines is attracting some attention.

HISTORICAL

Bose (6) has studied some of the wood rots in forest areas in Bengal. Butler (10) refers to a disease, "Betelnut Plague," in Sylhet, apparently caused by *Fomes lucidus*. The earliest reference to the betelnut plague is by Watt (46), who says that it was reported to him in March, 1896; he records evidence to show that it appeared in November, and December, 1892. Petch (31) states that Berkeley's record of *Polyporus lucidus* (Leys.) Fr. is from Gardner's collection of 1846 from Ceylon. Since the conditions in India are not far different from those in Ceylon, it may be presumed that this fungus was present in India at about the same time. Petch (31) collected *Fomes lucidus* (Leys.) Fr. from 1909-1915 from various parts of Ceylon. In 1913 Butler (13) refers to the finding of *Polyporus* (*Fomes*) *lucidus* on diseased areca and cocoanut palms in Mysore and Ceylon.

The first reference to the disease on areca palms, *Areca catechu* L., in Mysore, "Anabe roga of supari" (the arecanut is also known as betelnut or supari), is by Coleman (17). He obtained cultures of the fungus by placing sterile bits of diseased stem in Roux tubes with distilled water, and after the mycelium had grown out, transferring the pieces to Erlenmeyer flasks containing ground areca root dust moistened with plum decoction. He got fructifications in some of the flasks that had typical basidia and basidiospores. He did not culture the fungus on agar. A few experiments on the control of the disease in the field by trenching and liming were tried, but with no success. Work on this disease was started by the writer in the year 1928, with a view to obtaining some insight into the life history of the fungus and to utilize it, if possible, in the control of the disease.

"Anabe-roga" literally means a disease caused by "Anabe" or a mushroom-like structure, which term is applied indefinitely to any fair-sized fungus. In this particular instance, the fungus is *Ganoderma lucidum* (Leys.) Karst.

HOSTS AND SYNONYMY

The fungus *Ganoderma lucidum* was first described under the name *Fomes lucidus* (Leys.) Fr. It is largely a tropical fungus, but it occurs also in the temperate regions. This fungus or one or other of its synonyms occurs in India, Burma, Ceylon, the Federated Malay States, the Dutch East Indies, New Guinea, Africa, and the West Indies in the tropics, and in the temperate regions of Europe and the United States of America. Butler (12) has described the fungus in detail. Butler and Bisby (15) cite as synonyms:

Fomes lucidus (Leys.) Fr., *Polyporus lucidus* Fr., *Ganoderma resinaceum* (Boud.), *Polystictus egregius* Massee, n. sp. (?), *Ganoderma amboinensis* (Lam.) Pat., *Fomes amboinensis* (Lam.), *Polyporus amboinensis* Fr.

According to Van Overeem (42), the following are synonymous with this fungus:

Ganoderma sessile Murrill, *Polyporus fulvellus* Bres., *P. resinosus* Schraeder, *P. curtisii* (Berk.) Murrill (after van der Bijl), *Ganoderma mangiferae* (Lév.).

The list of hosts recorded for the fungus from various parts of the world comprises about 44 species belonging to 34 genera from 18 families. Out of these the family Leguminosae alone is represented by about 11 genera and 17 species; so that Novell (29) is partly justified in saying that "there is some cumulative evidence of its parasitism on leguminous trees." Sharples (34) has recorded *Polyporus ostreiformis* on arecanut and coconut palms in Malaya, and McRae (27) has found the same fungus on arecanuts in Calcutta. In Mysore the writer has noticed *Ganoderma lucidum* on *Areca catechu*, *Cocos nucifera*, *Cassia siamea*, and *Pongamia glabra*. An exudation, similar to that that takes place in these hosts affected by the fungus also has been noticed in *Artocarpus integrifolia*. No sporophores were obtained, and no determinations could be made. This study is chiefly on *Ganoderma lucidum* occurring on *Areca catechu* Linn. in Mysore.

ECONOMIC IMPORTANCE

The disease is found to be rather severe in gardens where the cultivation has been neglected, or where there is a tendency for water to stagnate due to deficient drainage. In one case it was very severe in a garden where *Saccharum spontaneum* was allowed to grow rather wild. Watt (46) also has noticed the betelnut plague to be severe in neglected gardens. The disease usually attacks trees from about 10 years of age and upwards.

Younger trees have not been noticed to suffer from it. The losses from this disease have not been correctly estimated. From reports given by the garden owners, it is estimated that about 15 to 20 trees die annually in an acre of 400 trees. If not attended to properly and if regular replantings

are not made, the annual losses may mount up to 50 to 60 trees per acre, until whole gardens are wiped out. Butler (10) says that "the actual loss suffered amounts in many cases to more than Rs. 75/- an acre annually, while some gardens were seen in which fifteen-sixteenths of the trees had been killed."

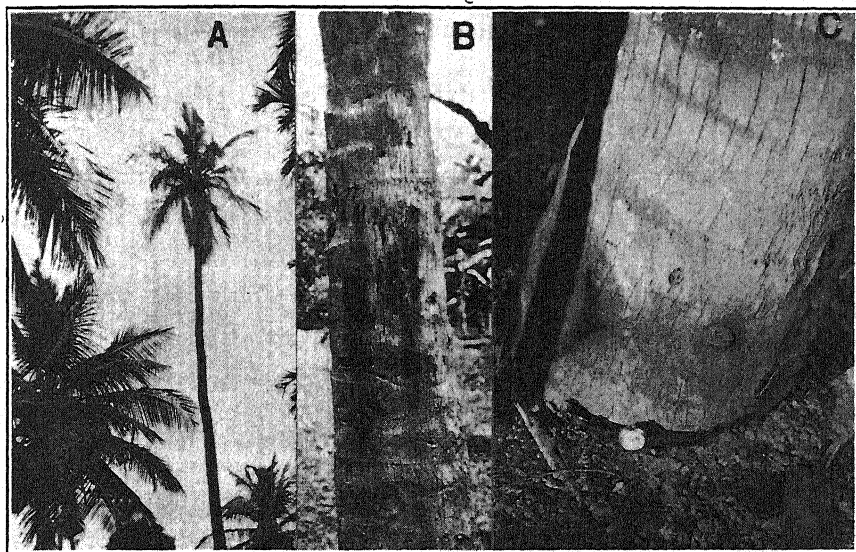


FIG. 1. A. Cocoanut palm showing narrowing of the crown and drooping of the basal leaves, the effect of ganoderma infection; healthy palms on either side. B. Another cocoanut palm trunk showing the profuse bleeding from numerous spots due to the same infection. C. Cocoanut palm inoculated with pure culture of *Ganoderma lucidum* on cocoanut showing bleeding 6 months after inoculation. The hole was plugged with cork after inoculation. The watch lying on the ground indicates the size of the inoculation hole.

SYMPTOMS

The symptoms of the disease on areca palms have been defined by Watt (46), Butler (10), and Venkata Rau (43), and for the disease on cocoanuts by Petch (32) and the writer (44). They are akin to those of drought. There is first a drooping or yellowing of the lower leaves, followed sometimes by a reduction in the size of the crown (Fig. 1, A). In later stages the crown dries up. In contrast to this, Watt (46) found the innermost leaves to be affected first, the crown of leaves separating from the trunk, and a foul smell in the bud. The ultimate effect, according to him, was to leave dead stumps behind until "what was once a plantation looked like a harbour with thousands of masts." Again, in contrast to the writer's experience, Butler (10) states that one of the earliest signs of this disease is a drooping of the

nuts, as in the Mysore disease, referring to "Koleroga" due to *Phytophthora* described earlier in his paper. The experience in Mysore is that affected trees continue to bear a crop for some years, even though it may be slightly less than normal. The observations of Watt and Butler lead one to suspect that they perhaps had also under notice some cases of top rot due to *Phytophthora arecae*. Indeed, Butler (10) says "the final appearance is just the same as in Koleroga." Additional support for this view is lent by the fact that Watt (46), himself, cites N. G. Mukharji's report, which records the latter's finding of a species of *Pythium* in diseased palms, though they could not get it a second time.

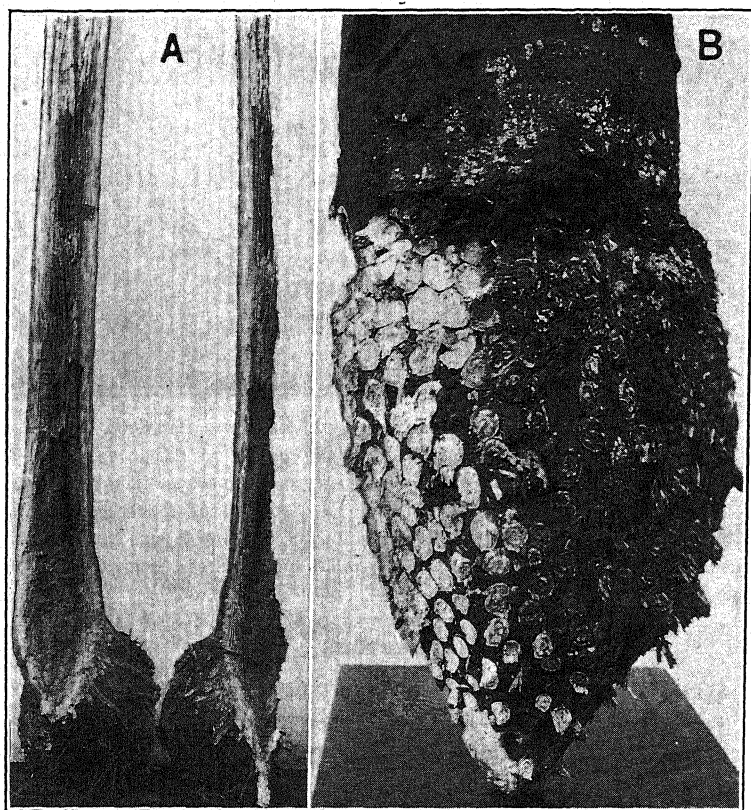


FIG. 2. A. Stump of areca palm cut open, showing late stage of ganoderma infection. The roots are all affected. B. Stump of areca palm showing the browning of the roots (right half) at an early stage of infection. Those on the left are yet unaffected.

The seat of the trouble, however, is at the base of the trunk and the roots. Affected roots are discolored, dry, and brittle (Figs. 2 and 3). From the lower portions of the trunk there is an exudation of a brownish gummy juice

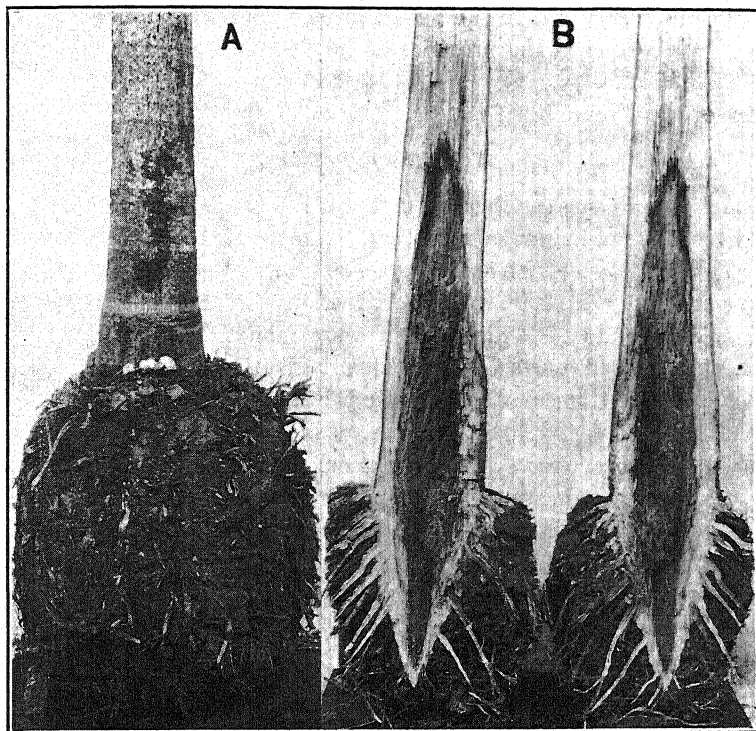


FIG. 3. A. Stump of areca palm showing the characteristic bleeding, and three sporophores at ground level. B. Stump of areca palm cut open to show the rotting inside. (Earlier stage than in figure 2, A.)

(Fig. 3, A), which is more copious in the cocoanut (Fig. 1, B) than in the arecanut. Some time after the exudation starts, fruit bodies of the fungus begin to develop (Fig. 3, A). Sometimes the fructifications appear on the stumps only after the cutting down of the palms. No stalk has been noticed in the fructifications on either the arecanut or cocoanut palms.

On cutting open an affected areca palm, the interior of the stem is found to be dark brown to about 2 to 3 feet from the ground (Fig. 3, B). The affected tissues emit a musty smell, very commonly associated with freshly growing sporophores of the Polyporaceae. On the outer border of the brownish zone is a yellowish zone, beyond which is the apparently healthy white portion. The brownish discoloration also extends down into the central thickened portion of the root system, and into the affected roots (Figs. 2, A, and 3, B). They are sometimes mottled with white on a brownish background.

On microscopic examination of the stem, the fungus was found both in the brownish and the yellowish regions, but not in the white region. It was

noticed in the vessels and in the xylem parenchyma, but not in the phloem. Affected vessels showed the presence of tyloses. Tyloses have been seen in plague-affected areca palms by Watt (46) and Butler (14). Watt attributed the cause of the disease itself to tyloses.

SECONDARY ORGANISMS

Besides *Ganoderma lucidum*, in many of the gardens visited, some of the affected trees showed signs of infection of the stems by *Thielaviopsis paradoxa*. This was evidently a saprophyte, as in the cases noticed it was found to gain entry only through wounds. Instances of trees with *Ganoderma* infection, alone, and no *Thielaviopsis* were many. *Thielaviopsis* appears soon on the exposed parts of palm stems. Butler (11) has found *Thielaviopsis* on palm tissues of several kinds very frequently, and is inclined to view it as a perfectly harmless fungus in India. Sundararaman *et al.* (40) have described successful inoculation experiments with *Thielaviopsis paradoxa* on areca palms, but only through wounds, and are themselves inclined to believe it is only a facultative parasite. Thompson (41) says in regard to the oil palm, *Elaeis guineensis*, that *Thielaviopsis* is the commonest mould, appearing in 36 hours on freshly pruned leaf bases, and that it is always present on diseased palm tissue, and, under certain circumstances, is definitely parasitic.

MATERIALS AND METHODS

The materials for this investigation were obtained from Amrutur, a village about 56 miles from Bangalore, where the arecanut is grown as a crop in gardens irrigated in summer from a tank.

Cultures of the fungus were obtained by transferring aseptic pieces of the tissue from near the margin of the apparently healthy and infected tis-

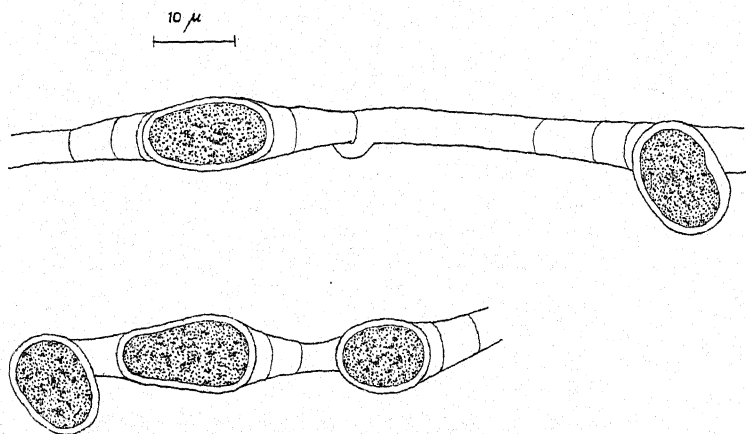


FIG. 4. Chlamydospores in pure culture on malt agar of *Ganoderma lucidum* on areca.

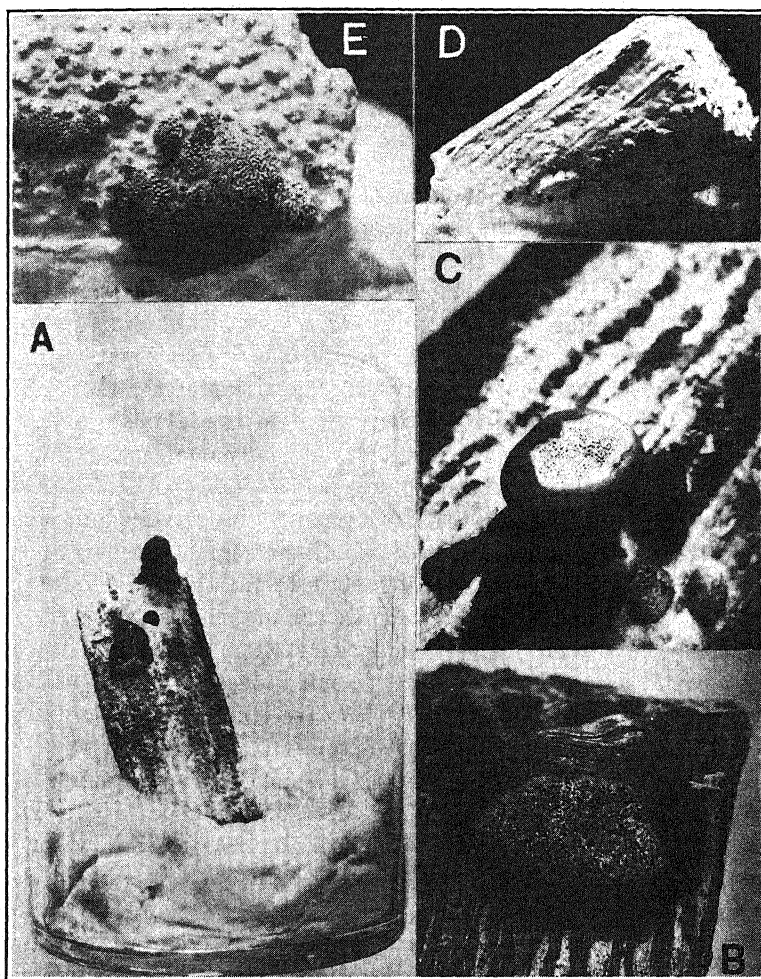


FIG. 5. A. Culture on areca wood of *Ganoderma lucidum* on areca, in bottle. Note thick mycelial mat on the wood, and spores on the mat dropped from the sporophore just under the pores. B. Another sporophore from culture on wood of the areca strain in bottle, showing the pores. C. Resupinate sporophores in culture on areca wood of the areca strain in bottle. D. Culture on coconut wood of *Ganoderma lucidum* on coconut, showing numerous origins of sporophore in the form of poriferous layers. E. Some poriferous layers from D enlarged.

sues into prune-juice agar and malt-agar slants. On the third day a hyaline mycelium grew out of the pieces, and subsequent transfers gave pure cultures.

THE FUNGUS

Cultural Characters.—The fungus grows slowly on malt and prune-juice agar. The subcultures show any appreciable growth from the inoculum only on the 5th day. In about 2 weeks the surface of the slant is covered with a thick flocculent growth. This appearance is partly due to the deposit of calcium oxalate crystals on the hyphae. The crystals are more prominent on growths in malt agar than those in prune-juice agar. There are both thin and thick hyphae. Clamp connections are more abundant on the thicker hyphae than on the thinner ones. The hyphal mat thickens on growth on agar, and more so on pieces of stem tissue of the arecanut (Fig. 5, A). The mycelium remains hyaline except in the later stages on pieces of stem, when it takes on a brownish tint. In only one case was a slightly yellowish tint noticed on a sterile hump in a culture bottle. The fungus forms chlamydospores (Fig. 4). They are mostly intercalary and sometimes terminal. Snell (38) mentions Faull's finding of chlamydospores in *Fomes officinalis*. Besides this fungus, Snell (39) found them also in *Trametes serialis*, *Lentinus lepideus* and to some extent in *Lenzites saepiaria* and *L. trabea*. They have been observed by Fritz (24) in *Polyporus sulphureus*, *P. balsameus*, *P. borealis*, *P. schweinitzii*, and *Polystictus versicolor*, and by Brefeld (7) in *Polyporus destructor*, *Fistulina* sp. and *Oligoporus* sp. No oidia have been observed in *Ganoderma lucidum*.

Sporophore Production.—The fungus did not form sporophores on agar, even though grown in big tubes 7"×1", containing about 15 to 20 cc. of medium. Neither exposing these tubes to light near a window for 1 to 2 hours daily, nor keeping them always in the light or always in the dark, in the vertical or horizontal position resulted in sporophore formation. A poriferous layer containing spores was, however, observed in the strain from the cocoanut palm, both on agar and on sterile pieces of cocoanut trunk in bottles (Fig. 5, D and E). Bose (5) succeeded in obtaining loose pores without any pilear form, but containing spores of *Ganoderma lucidum* on agar. He sent a subculture of his isolation to Dr. Coleman, which on sub-culturing did form an irregular cup-shape structure bearing the hymenium and spores, somewhat similar to the poriferous layer formed by the cocoanut strain. Bose (6) has observed such structures containing only spores and numerous crystals of calcium oxalate in *Trametes gibbosa*, *Polyporus adustus*, *P. ostreiformis*, etc.

With a view to getting the fungus to produce sporophores, the method adopted by White (48) was employed. Pieces of areca stem were used from

which the air had been driven out by alternately boiling and plunging into cold water 3 times. The blocks were about 3" x 2" x 1", and were placed in a slanting position in 1-litre wide-mouth bottles on wads of absorbent cotton about 1" thick, and thoroughly moistened. The bottles were autoclaved at 1 atmosphere pressure for 20 minutes, and used for inoculation after 2 weeks. Out of 8 bottles inoculated, sporophores developed in 6. Regular sporophores developed in 3 bottles, and in a fourth the sporophore remained in an incipient stage, probably owing to the drying out of the cotton. In 2 other bottles the sporophores failed to develop spores. The sporophores were of the bracket type, only smaller (Fig. 5, A and B), as was to be expected, seeing that the available nutriment was so small. Sometimes they developed resupinate fashion (Fig. 5, C). Spores resembling natural ones dropped down to the bottom of the bottles.

In a month from the time of inoculation, drops of a colorless juice were seen on the mycelial mat covering the pieces of stem. These were sometimes seen also in the culture tubes. Bose (6) has noticed a juice in *Ganoderma lucidum* and *Trametes gibbosa*. In the latter he observed the drops to contain numerous round, white and brownish conidia, both of approximately the same size, by transferring which to agar he recovered the mycelial growth of *T. gibbosa*. No conidia were seen by the writer in the drops secreted by *G. lucidum*. The earliest signs of sporophore formation occurred on the 72nd day. Three humps were noticed that gradually developed a deep lac color, as in the natural specimens (Fig. 5, A), and in seven weeks from that time, spores were seen to shed from one of the humps at the side. In a second bottle the sporophore formed 3 days later than in the first case, pores were seen to form 2 weeks later, and spores began to fall about 5 weeks from the appearance of the pores. Hence, under the conditions prevailing in the bottles, the pores developed in about 3 months from inoculation of the pieces, and spores began to fall in about a month from the appearance of the pores.

The bottles were kept in a closed cupboard and were not exposed to light, except to the diffused light of the room for purposes of routine inspection. According to Miss Ames (1), Miss Bayliss obtained only abortive fruit bodies in the absence of light, which Miss Ames attributed to the probably abnormal laboratory conditions. White (48) failed to get the typical bracket form of fructifications in his bottle cultures. Long and Harsch (26) succeeded in getting typical pilei in artificial cultures on agar when light was made to fall parallel to the tubes irrespective of whether these were placed vertical or horizontal. They found from their study of wood rotting fungi that many *Polyporaceae* produced sporophores in diffused light, others required direct sunlight, while some produced sporophores in absolute darkness like *Polyporus farlowii*, *P. cinnabarinus*,

and *Trametes serialis*. *P. cinnabarinus*, unlike the other 2, produced only sporophores but no spores. They found that the character of the substratum played only a minor part in sporophore formation. Bose (6) found that the direction of the rays of light had no influence in the development of typical pilei by *Trametes lactinea*.

SPORE GERMINATION

Various media were tried for germination of spores, such as malt agar, prune-juice agar, distilled water, tap water, rainwater, 1 and 2 per cent malic and citric acids, and Buller's gelatine medium (9). In no case was germination observed. Both material from the field and from artificial culture gave negative results. Coleman (18) also failed to germinate the spores, and he believed that the failure might be due to the chitinous endospore, because spores with thin undifferentiated walls like those of *Pomes fomentarius*, germinate readily. Bose (5) got germination of the spores of *Ganoderma lucidum* on 3 per cent malt-extract agar of pH 6.9.

DECAY

Since decay is essentially an enzyme action, an attempt was made to study the enzymes present in *Ganoderma lucidum*. Buller's work (8) on *Polyporus squamosus* showed that this fungus secreted laccase, tyrosinase, amylase (diastase), emulsin, protease, lipase, rennetase, and "coagulase," and possibly cytase and hadromase, all except the last two in the sporophores.

Schmitz and Zeller (33) found that the following enzymes were present in *Polyporus lucidus* viz., esterase, maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, emulsion, tannase, urease, and trypsin and erepsin when fibrin is used as a substrate.

Nutman's work (30) on *Polyporus hispidus* showed that the fungus secretes emulsin, diastase, invertase, ligninase, hemicellulase, oxidase, and catalase.

In this work on enzymes Nutman's (30) method of growing the fungus on liquid extracts and testing for the enzymes in the mycelium was followed. The extracts used were of turnip, beet-root and carrot. Two hundred and fifty grams of each sliced thin were added to 500 c.c. water and boiled in the autoclave at 115° C. for 20 minutes, cooled, filtered, each put into 5 Erlenmeyer flasks and autoclaved at 115° C. for 20 minutes. The flasks were inoculated with 4 pieces each of inoculum from malt-agar culture. Only 1 of the carrot decoction flasks got contaminated and was discarded. The surface of the liquid in the flasks of turnip extract was covered by the fungus growth in from 10-15 days, whereas that of carrot was filled in 20 days, during which time the beet-root extract was only half-covered.

Thus, as Nutman found, turnip extract is the best medium for the growth of the fungus. The cultures were kept at laboratory temperature. After 8 weeks, the mycelial mats were washed and dried between blotting paper and then over calcium chloride *in vacuo* for a week. The enzyme extract was prepared by grinding the dried mycelial mat with an equal weight of silver sand in an agate mortar and steeping it in distilled water, 20 parts to 1 of mycelium for 24 hours. The extract was filtered and toluene added as an antiseptic. The methods were adapted from Buller (8) and Nutman (30).

ENZYME TESTS

Diastase

One c.c. of the enzyme extract was added to 20 c.c. of a 5 per cent solution of soluble starch in a test tube. To another tube containing the same amount of starch, 1 c.c. of the boiled extract was added and to a third tube 1 c.c. distilled water. The three tubes were tested after a day with Fehling's solution. The first tube to which the unboiled extract was added showed reduction, while the other two did not. Hence *Ganoderma lucidum* secretes diastase.

Laccase

To 10 c.c. of a 1 per cent solution of hydroquinone in a flask 1 c.c. of the enzyme extract was added and to another flask of the same solution 1 c.c. of the boiled extract. In the flask to which the enzyme extract was added a brownish red color developed after 24 hours, which deepened after 48 hours. There was a green iridescent pellicle on the surface of the liquid, as observed by Buller (8). The control gave no change, even after 8 days. This shows that the fungus secretes the oxidase, laccase.

Protease

To a solution of 7 per cent nonneutralized gelatine in 2 test tubes, 2 c.c. of extract was added to the one and 2 c.c. of the boiled extract to the other. A little toluol was added to each tube as an antiseptic. After 24 hours the gelatine was liquefied in the first tube, but not in the second. The gelatine was liquefied to a depth of 6 mm. after 48 hours and a further 3 mm. after another 30 hours. The control was unaffected. This experiment shows that the fungus secretes a protease.

Rennetase

To 10 c.c. of fresh milk in a test tube, 1 c.c. of the enzyme extract was added and to another tube of milk 1 c.c. of boiled extract. Toluol was added to both tubes as an antiseptic. The tube containing unboiled extract

showed coagulation after 5 hours, while the control showed no change, showing that the mycelium secretes rennetase.

Lipase

To 40 c.c. of a 1.84 per cent ethyl acetate in each of 2 flasks, 2 c.c. of extract was added to one and 2 c.c. of boiled extract to another. One-half c.c. toluol was added to each flask. The flasks were corked and placed on top of a paraffin imbedding bath, where the temperature ranged from 42° C.-45° C. Ten c.c. portions were taken from both flasks after 5 days and 7 days and titrated with N/20 KOH. In both cases, on both occasions, the experimental portion required less alkali for titration than the control, instead of requiring more, which should have been the case if there had been any hydrolyzation. This shows that the fungus does not secrete lipase.

Invertase

To 8 c.c. of 1 per cent cane-sugar solution in each of 3 test tubes was added 1 c.c. of enzyme extract to one tube, 1 c.c. of boiled extract to a second, and 1 c.c. of distilled water to the third. After 24 hours there was a very faint reduction in the first tube, which increased slightly after 48 hours, but there was no reduction in the other two. But that it is able to utilize cane sugar is evident from the fact that in the solutions used in the H-ion concentration studies described later, the fungus develops a thick mycelial mat. *Ganoderma lucidum* seems to secrete small quantities of invertase, as was found to be the case by Nutman (30) in *Polyporus hispidus*.

Maltase

To 100 c.c. of 2 per cent maltose in each of two 125 c.c. Erlenmeyer flasks, 1 c.c. of extract was added and the contents of one of the flasks boiled. After cooling, 1 c.c. toluol was added to each flask and the flasks were stored at room temperature (about 25.5° C.). After 3 days 25 c.c. from each flask were removed and mixed with basic lead acetate and filtered. The clear solutions were tested in the polarimeter. The readings were the same in both solutions, indicating that the fungus does not secrete maltase.

Coagulase

A 5 per cent solution of Lintner's soluble starch was made, boiled, and filtered. The filtered solution was put into wide test tubes 7" x 1". To one of the tubes 1 c.c. of the enzyme extract was added, and to the other 1 c.c. of the extract, boiled. To both tubes 0.5 c.c. toluol was added as an antiseptic. After 15 hours a cloudy precipitate 1" high was noticed at the top of the first tube, but none in the second. After about 90 hours

the precipitate gradually settled down in the first tube and the second showed no change. The next day the clear solutions were tested with iodine; that from the first tube gave a violet reaction, whereas the one from the second tube gave a blue reaction. This experiment proves that the fungus secretes coagulase.

Catalase

Presence of this enzyme was tested by adding the enzyme extract, the boiled extract, and distilled water to hydrogen peroxide. There was no frothing or effervescence in any case. This shows that catalase is not secreted by the fungus.

Oxidase

For the determination of oxidase Bavendamm's method (3) of growing the fungus in agar to which tannic acid had been added was tried. The medium was potato-dextrose agar in Petri dishes, and the percentages of tannic acid tried were 0.25, 0.5, 1, 1.5, 2 and 3 per cent, respectively. The

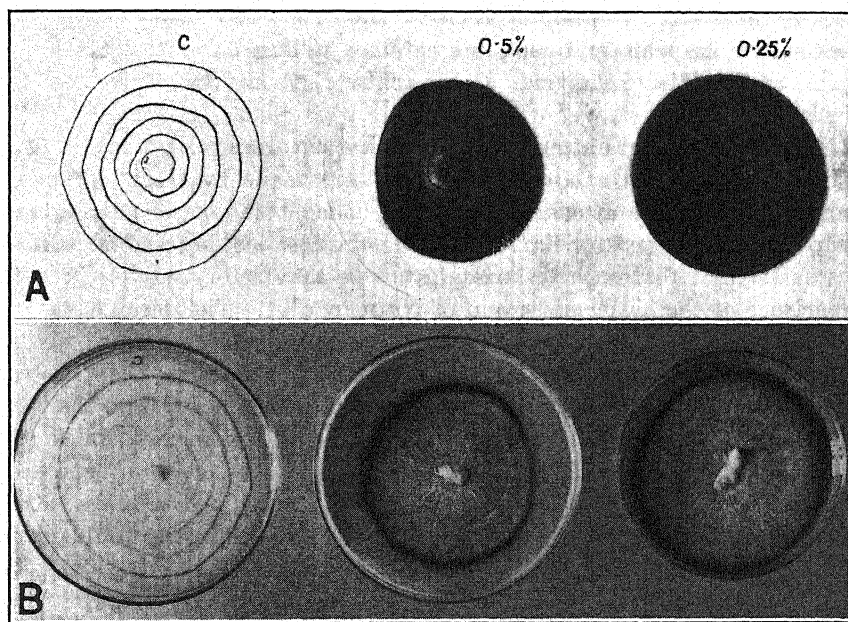


FIG. 6. Petri-dish cultures on potato-dextrose agar of *Ganoderma lucidum* on areca to demonstrate the production of oxidase. A. Bottom view of dishes showing the limit of growth from third to eighth day. B. Top view. Both series from left to right, check (c), 0.5 per cent tannic acid, 0.25 per cent tannic acid. In the latter two cultures the brown halo around the fungus is prominent and can be seen in advance of the mycelium.

agar to which 0.25 and 0.5 per cent tannic acid was added set, while the others did not. The fungus grew well in plain potato-dextrose agar, and in 0.25 and 0.5 per cent tannic acid potato-dextrose agars, but there was no growth in the others. The brown halo in the tannin cultures was quite noticeable, even on the second day and kept on increasing from day to day in advance of the mycelium. (Fig. 6). This experiment shows that the fungus secretes oxidase and is thereby able to utilize tannin. Since tannin is analogous to lignin it may be presumed that the fungus is capable of destroying lignin. Viala (45) has found that *Fomes igniarius*, causing apoplexy of the vines, destroys tannin by means of an oxidase in the dead wood, which acts on the tissues in advance of its growth. He also found that active growth occurs only in tissues rich in tannin, and the attack occurs usually on vines after they are about 15 years old, before which treatment is not required. This may possibly account for the fact that ganoderma infection has not been noticed on very young arecanut or cocoanut palms.

These enzyme studies have shown that the fungus secretes diastase, laccase, invertase, protease, coagulase, rennetase, and oxidase, and that it does not secrete maltase, lipase, and catalase. Thus it secretes both hydrolysing and oxidising enzymes as shown by Nutman (30) for *Polyporus hispidus*.

In the early stages attempts to study the nature of the decay in areca palms attacked by *Ganoderma lucidum* were made by means of microchemical tests. The attacked tissues responded both to the phloroglucin-hydrochloric acid test for lignin and the chlor-zinc-iodide test for cellulose. It was thought that such tests would not be satisfactory, as has been the experience of the biochemists, and of Nutman (30). The decay caused by *Ganoderma lucidum* on areca palms is of the brown-rot type, which, according to Falck and Haag (23) and Bavendamm (3), would be the "destruction" type. Since *G. lucidum* can use tannin, it would fall into the "corrosion" group, unless it may be one of the transitional forms. Falck (22) has found that *Fomes annosus* causes a decay of the corrosion type characterized by the simultaneous disintegration of lignin and cellulose. Only detailed chemical analyses of the sound and decayed tissues would give a correct idea of the nature of the decay.

H-ION CONCENTRATION

That soil acidity favors the growth of wood-destroying fungi has long been recognized. Wolpert (49) has summarized the literature on this subject. He found that wood-destroying fungi grew best on acid solutions and that their growth increased the initial acidity of the solutions. Anderson (2) found that soil acidity is the dominant predisposing cause in the

attack of *Fomes annosus* (*Trametes radiciperda*). This view has been supported by Weis and Nielsen (47). Curtin (19) and Curtin and Thordarson (20) found acid production in a number of fungi. Curtin (19) opines that acids considerably assist in the liberation of carbohydrates that serve for the nutrition of wood-rotting fungi. Campbell's work (16) on the chemical aspect of wood rots has led to the conclusion that acid production and enzyme action are closely related. He suggests that acids might be formed by the action of an oxidase on lignin and pentosans and then these react together with the oxidase to bring about the later stages of decay in which cellulose is decomposed.

Wolpert's method (49) was followed to study the growth of *Ganoderma lucidum* in relation to H-ion concentration. Two culture solutions were used, modified Richard's E solution, and peptone nutrient solution with sugar. The pH readings were taken with the aid of a Hellige comparator, and only double-distilled water was used in preparing the solutions. Precautions regarding glassware suggested by Wolpert (49) were taken and pipettes were dry-sterilized at 150° C. for one hour. The cultures were kept at laboratory temperature. The mean temperature during the time of the experiment varied from 22.5° C. to 30° C. Observations were made upon the final pH and the dry weight of the fungus in the manner suggested by Wolpert. The inoculum was prepared by growing the fungus on agar according to Zeller, Schmitz, and Duggar (50), as recommended by Wolpert. To 1000 c.c. of potato water (from 200 grams of peeled and sliced potato cooked for 30 minutes in the autoclave at 15 lbs. pressure) were added 20 grams of cane sugar 10 gms. KNO_3 , 5 gms. of KH_2PO_4 and 20 gms. of agar. Eight m.m.-square pieces of inoculum were used, one to each flask.

Modified Richard's E Solution

This solution contained: MgSO_4 , 0.5 gm.; KNO_3 , 5.0 gm.; NH_4NO_3 , 10.0 gm.; Cane sugar, 50.0 gm.; FeSO_4 , trace; and varying amounts of H_3PO_4 , KH_2PO_4 , and K_2HPO_4 to give a total of 10.4 gms. of phosphate; and double-distilled water 1000 c.c. Its composition was adjusted as recommended by Wolpert (49). A total of 35 c.c. of solution was used in 100–120 c.c. Erlenmeyer flasks. Three flasks of each grade of pH were prepared, one being used to verify the pH and the other 2 for growing the fungus in duplicate cultures. In a few instances owing to the necessity of adding the phosphate solutions after sterilization, contamination resulted in one of the flasks which was rejected, and the weight of the mycelium taken from only one of the flasks. Otherwise, the weights given are the average of the two cultures. There was a slight variation in the initial pH as given by Wolpert (49) and as ascertained by the Hellige comparator,

and to show these comparatively, Wolpert's figures are given alongside in the tables. The dry weights were taken at the end of 7 weeks from the date of inoculating the cultures.

TABLE 1.—Showing the growth of *Ganoderma lucidum* in modified Richard's E. Solution of different pH

pH			Dry wt. of mat in mgs.
Wolpert	Initial	Final	
3.0	3.4	2.8	6.6 ^a
3.5	3.8	2.8	12.2
3.9	4.0	2.8	12.8 ^a
4.4	4.5	2.8	8.1
5.0	4.5	2.8	17.8
5.5	5.4	2.8	34.0
6.0	6.0	4.1	23.0 ^a
6.5	6.6	4.3	93.5
7.0	7.0	4.5	45.1
7.6	7.4	6.8	only very faint growth
7.8-8.2	7.8-8.2	no growth

^a Only 1 culture, the others 2.

It is seen that in modified Richard's E solution the best growth of the fungus is obtained at pH 6.5 with a weight of 93.5 mgs., with a secondary maximum at pH 5.5 with 34 mgs. of mycelium. The fungus grows at pH 3.0, and even at pH 7.0 the mycelium attains a weight of 45.1 mgs. But on the alkaline side there is a sudden drop, the fungus barely showing any growth at pH 7.4 and none at all at pH 8.0. In all the solutions the fungus increased the initial acidity.

Peptone Nutrient Solution with Sugar

This solution contained Witte's peptone, 25 gms., cane sugar, 30 gms., MgSO_4 , 0.5 gms., FeSO_4 , trace, varying amounts of H_3PO_4 , KH_2PO_4 and K_2HPO_4 to give a total of 9.65 gms. of phosphates, and double-distilled water to make 1000 cc. of solution. The dry weights of mycelium were taken 6 weeks from the date of inoculation.

The peptone solution with sugar was the better of the two solutions used, the poorest growth in this solution being better than the best growth in modified Richard's E. solution. The fungus grows best at pH 6.6, with a weight of 300.3 mgs. of mycelium. There are secondary maxima at pH 5.1 and 4.1 with weights of mat of 235.5 mgs. and 215.1 mgs., respectively. The fungus grows at pH 2.8 with mycelium weighing 95.1 mgs. It grows at pH 7.0, but here again, as in Richard's E solution, there is a sudden decline in growth

TABLE 2.—*Growth of Ganoderma lucidum in peptone nutrient solution with sugar of different pH*

Wolpert	pH		Dry wt. of mat in mgs.
	Initial	Final	
2.0	2.0	2.0	no growth
2.5	2.8	2.8	95.1
3.0	3.7	2.8	157.4
3.5	4.0	2.8	202.9 ^a
3.9	4.1	2.8	215.1
4.5	4.5	2.8	150.4 ^a
5.0	5.1	2.8	235.5
5.6	5.7	2.8	159.0
6.0	6.2	6.0	169.0 ^a
6.5	6.6	5.5	300.3 ^a
7.0	7.0	6.0	130.2 ^a
7.4	7.7	7.6	trace ^a
7.8–8.2	7.9	7.7	trace ^a
8.5–8.7	8.5	8.5	no growth ^a

^a 2 cultures, the others 3.

on the alkaline side. In peptone solution also the fungus tends to increase the initial acidity. Wolpert (49) failed to notice an increase in acidity in this solution for the fungi he studied, except for *Lenzites saeppiaria*, which alone increased the acidity throughout the entire pH range and at all temperatures.

The tendency noted by Wolpert (49) of a wider optimum range of pH in the more favorable solution, with a slightly fluctuating optimum zone covering several pH units is noticed also for this fungus. In the peptone solution the fungus shows good growth between pH 3.0 and 6.5, whereas in Richard's E solution the range is only between pH 5.0 and 7.0. In the case of Richard's E solution the extreme limit of the acid scale was not determined. This, in the peptone solution, is pH 2.0, which does not support the growth of the fungus. On the alkaline side pH 7.6 does not support growth in either of the two solutions.

It is clear that *Ganoderma lucidum* secretes acids during its growth.

PARASITISM

The bracket fungi have been considered to be wound parasites. The inoculation experiments with fungi of this type on living trees have not been very successful. White (48) has reviewed the literature on parasitism of the wood-destroying fungi. Since then, Baxter (4) and Nutman (30) have obtained some amount of success in inoculations with *Polyporus hispidus* on

ash trees. They have found the fungus growing in the tissues to some distance from the inoculated points.

Shaw (36) failed to get successful inoculations on sal trees, *Shorea robusta*, with *Polyporus shoreae* Wakef. He (35) reports successful inoculations by Hafiz Khan on Guazuma with *Fomes lucidus* (Leys.) Fr.

Gadd (25) states that inoculations with *Fomes lucidus* on two hevea trees have resulted in no ill effects.

Thompson (41) considers *Ganoderma lucidum* and *Fomes applanatus* to be only of subsidiary importance in bringing about infection on the oil palm, *Elaeis guineensis*, in Malaya, when considered in relation to *Fomes* sp., he isolated from diseased trees, which he thinks probably is related to *F. pachyphloeus*.

Nojima (28) has succeeded in inoculation experiments with *Polyporus japonicus* on oak roots in Japan.

De Jong (21) considers *Rigidoporus microporus* (*Fomes lignosus*) to be a weak parasite on rubber in the Dutch East Indies. Out of 21 trees he inoculated, 3 died within 2 years from inoculation.

The contrary view that in the suspected cases of parasitism of the Polyporaceae, *Rhizoctonia bataticola* is the organism concerned, has been advanced by Small (37) but has had little support from others.

The little success that has attended experiments in this direction is probably due to the slow-growing nature of the parasites. As White (48) says "it is difficult enough to make an inoculation without contamination, but it is much more difficult to prevent subsequent contamination during the months or even years through which the test must run, and at the same time to be sure of maintaining the moisture and air factors suited to the fungus. Moreover, we are very uncertain as to the extent of the counteracting influence exerted by the host in fresh wounds, such as inoculation incisions should be."

Accordingly, in the inoculation experiments to be described, although only pure cultures were used as inoculum, the conditions under which the inoculations were made were such as not to exclude contamination of foreign organisms. If checks are provided in sufficient numbers, they would be equally liable to this foreign infection; hence such scrupulous attention to sterile conditions is perhaps unnecessary.

The first inoculation experiments were made on young palms grown in the laboratory garden at Bangalore with the inoculum in the form of agar cultures and *Cassia siamea* root cultures. They were unsuccessful.

Further work was done on full-grown palms in the garden of the Government Experimental Farm at Babbur, a locality where the disease had not yet put in its appearance. Inoculum was prepared by growing the fungus on bits of *Cassia siamea* roots in Roux tubes with distilled water or

on soaked cotton in big test tubes 7" x 1". The fungus was allowed to grow for 2 to 3 weeks before inoculation, and was vigorously growing at the time. The inoculations were made by carefully digging the soil around the roots, cutting a root with a scalpel, placing the inoculum close to the wound, and covering up again with the soil. In some cases the inoculum was placed in sample tubes 2" x 1" into which the injured root was introduced; in a few others the inoculum was wrapped round the injured root in a piece of cellophane paper. Where the tree was inoculated in more than one root, other roots at different sides of the tree were chosen. There were check rows in between every 2 rows of inoculated palms. In a second experiment 3 rows of check trees were left in between two infected rows so as to prevent the possible growth of the roots of check trees towards the inoculum and thus getting the infection. Fifty-five palms were inoculated in all, of which 25 received 1 piece of inoculum each, 8 received 2 each, and 22 received 3 pieces each. Similarly, in the check rows, some trees were wounded in 1 root, some in 2, and some others in 3 roots, while there were also some unwounded checks.

In about 5 months one tree in a check row adjoining an infected row showed a yellowing of the crown, and in another 3 months the crown was dried up. At about this time one of the inoculated trees also showed the drying of the crown. The trees were then cut down, and the stumps brought to Bangalore. Some of the roots in both the palms were dry and discolored, as in the natural infection, and the characteristic mycelium with the clamp connections was noticed in the roots. The discoloration was also noticeable in the butt of the palm, and a microscopic examination showed the fungus in the discolored portion. Further examination was made in the field one year after the inoculation. Out of 11 palms examined, the roots of 5 showed fungous infection, those of 2 showed a slight discoloration and those of 4 were healthy. Of the last 4, the crowns of 2 palms showed yellowing. It was not thought advisable to disturb the roots of the other palms at that stage.

In the second experiment, at the end of 5 months the crowns of 3 trees were yellowish, and the basal leaves of 1 tree were showing signs of drooping. The roots in some cases were dry and brittle and the fungus was seen to be actively growing. Besides these one palm had the inoculum tied by wire to 2 roots near the surface of the ground, after removal of an outer slice of tissue by means of a scalpel. In this case, at the end of 6 days the fungus was growing well on the cut surface of the root. At the end of 5 months both these roots had been exposed by the irrigation water. The inoculum had disappeared from one root, but the root itself was dry and friable. Its base was discolored, the discoloration proceeding to the heart of

the palm. The tissues about 4 inches away from the inoculation showed the fungus. The other root had come away with the inoculum. It was dry and the end of the root still attached to the tree was rotten.

Four cocoanut palms in the laboratory garden were inoculated in the same manner as above with the cocoanut strain, but into the trunk bored with a round chisel. Within six months a slight bleeding was noticed in an inoculated tree (Fig. 1, C) but none in the check. The fungus was seen to be growing in the tissues in all 4 palms.

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SUMMARY

Ganoderma lucidum, a very common tropical fungus, causes a disease of areca and cocoanut palms in Mysore and other parts of India. The fungus has been brought into culture from the infected wood, and, on inoculating sterilized pieces of areca palm tissue, has produced sporophores with true pilei.

A study of some of the enzymes present in this fungus has shown that it secretes diastase, laccase, invertase, protease, coagulase, rennetase, and oxidase, and that it does not secrete maltase, lipase, and catalase. The decay it causes is of the brown-rot or "destruction" type; but, since it is capable of using tannin in cultures, it is probably a transitional form, like *Fomes annosus*, which disintegrates lignin and cellulose simultaneously.

The fungus grows best at pH 6.5, but is capable of growth between pH 3.0 to pH 7.0. On the alkaline side there is hardly any growth. The growth of the fungus tends to increase the initial acidity of the solutions.

Ganoderma lucidum appears to be a very slowly active parasite. Large pieces of inoculum cultivated on bits of *Cassia siamea* roots were introduced near living trees. The fungus was found to be growing in the roots and producing discoloration in 5 months. In one case of an artificially infected tree, death resulted and the fungus was found in the tissues. One of the control trees also died, probably due to its proximity to the infected trees; and, similarly, the fungus was found to have permeated its tissues.

An inoculation into cocoanut palms with the cocoanut strain resulted in inducing bleeding symptoms as in natural infection and the fungus was found to be growing in the tissues.

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THE SYMBIONTS OF PSEUDOCOCCUS BREVIPES IN RELATION TO A PHYTOTOXIC SECRETION OF THE INSECT¹

WALTER CARTER

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INTRODUCTION

In a previous paper² attention was called to the fact that *Pseudococcus brevipes* (Ckl.) produced green spots on pineapple leaves at the points of feeding; and that this capacity was absent in mealy bugs from the roots of certain grasses but when present in the adult female mealy bug, was transmitted by her to her young. Since the symbionts of insects are similarly transmitted, the relationship of these organisms to the green-spotting capacity of the mealy bug has been studied with the object of checking the hypothesis previously set up² that an insect's symbionts conditioned its secretions. The fact that in this case the secretion is definitely phytotoxic adds significance to the results, since the majority of phytotoxic insects and virus carriers are sucking insects in which internal symbiosis is highly developed.

MATERIAL AND METHOD

Mealy bugs from heavily green-spotted pineapple plants were used. These were transferred in varying numbers and at various ages to panicum grass, *Panicum barbinode* Trin., growing in cloth-covered cages. After varying intervals of time, these mealy bugs or their progeny were returned to pineapple leaves for tests of their green-spotting capacity. Concurrent with these tests, examinations were made from time to time of the symbiont flora of individuals from the colonies of mealy bugs used. The method here was to dissect out the insect's mycetome, smear it rapidly on a slide, and, after a short period of air drying, stain with Heidenhain's iron-haematoxylin. Some 500 preparations were examined during the course of this study, and mealy bug colonies have been kept under observation for upwards of 2 years.

EFFECT OF THE HOST PLANT TRANSFER

A. On green spotting. When mealy bug colonies were allowed to feed for short periods of 10 to 14 days on panicum grass and then returned to pineapple leaves, the latter became green-spotted. Such experiments indicated that length of feeding time on the grass was a factor.

¹ Published with the approval of the Director as Technical Paper No. 81 of the Experiment Station of the Pineapple Producers Cooperative Association, University of Hawaii.

² Carter, Walter. The spotting of pineapple leaves caused by *Pseudococcus brevipes*, the pineapple mealy bug. *Phytopath.*, 23: 243-259. 1933.

The method of using gravid females was then employed. Numbers of gravid females were introduced into the cages containing grass in vials covered with cloth in such a manner that only the new-born crawlers could reach the grass. As checks on the green-spotting capacity of the colony, additional females were transferred to pineapple leaves. The colonies produced by these check females invariably produced spots.

A large number of small cages of grass, each containing 25 females, were set up in this manner. The numbers of young mealy bugs establishing themselves on the grass were very small. One effect of the host transfer was the immediate production of a large percentage of males that were useless for further study; another was to slow down the development of the insect so that from many of these cages only 3 or 4 specimens were obtained. The method was then used of transferring hundreds of gravid females direct to the stems of panicum in larger cages. From these it was possible to obtain an adequate supply of material.

In table 1 are summarized the data from 8 series of tests with the progeny of gravid females removed from green-spotting colonies to panicum grass. In 7 of these tests the green-spotting capacity survived in some individuals.

TABLE 1.—*Transfer of gravid female mealy bugs from pineapple to panicum grass. Summary of tests with first generation*

Date females transferred to panicum	Date progeny transferred to pineapple	Green spotting by progeny		Dissection of progeny	Condition of symbionts
		+	—		
11/15/33	1/1/34	1	2	12/14/33 1/22/34	Rod-shape form “
11/21/33	12/7/33	“
11/15/33	1/10/34	3	0	12/8/33 1/10/34	“ “
11/29/33	1/10/34	2	0	No record	No record
11/29/33	1/22/34	2	0	No record	No record
2/28/34	5/1/34	4	2	5/21/34	Rod-shape form
2/28/34	4/6/34	5	51	4/6/34 5/15/34	Coccus rods Rod-shape and intermediate forms ^a
2/20/34	3/20/34	0	100	3/20/34	Intermediate in 1; coccus rod in 9

^a 5/15/34 dissection was of green spotting individuals.

Evidence that green spotting in first-generation mealy bugs on panicum is limited to those individuals and not transmitted to their progeny is seen in figure 1 (left), which represents graphically the history of a colony car-

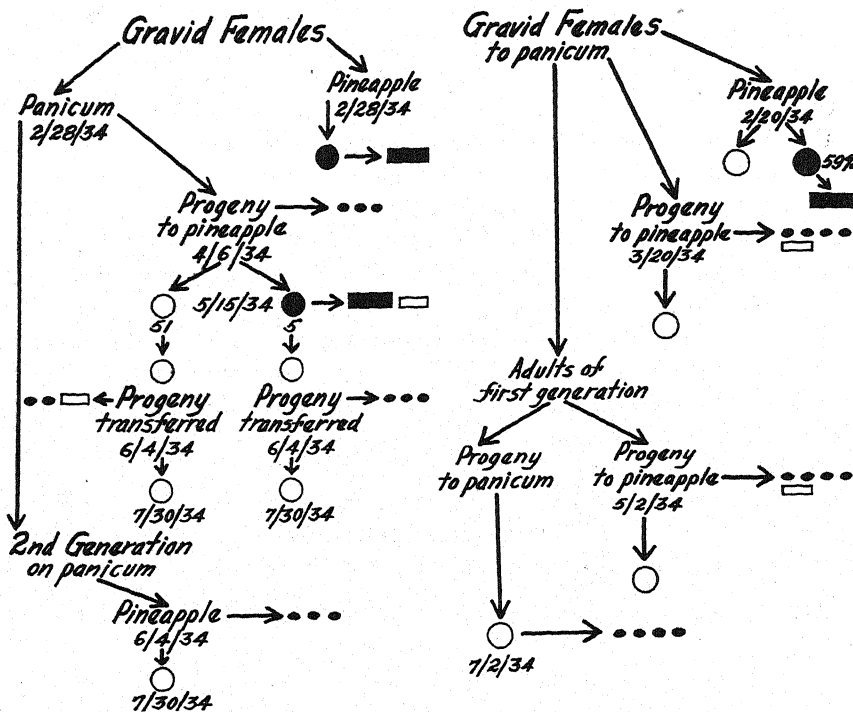


FIG. 1. Colony histories showing results of green spotting tests and the condition of the rod-shape symbiont. Legend: Black circle, green spots; hollow circle, no green spots; black bar, typical rod-shape form; hollow bar, intermediate form; oval, coccus rod.

ried to second generation on panicum grass. None of the second generation produced spots when returned to pineapple leaves.

Figure 1 (right) is a diagram of a colony history in which the green-spotting capacity was lost in the first generation. This was, no doubt, related to the fact that only 59 per cent of the mealy bugs in this colony were producing green spots when transferred to panicum, so that the colony as a whole responded more rapidly to the changed environment.

Continued growth of all these colonies on pineapple plants has failed to restore the green-spotting capacity.

THE SYMBIONTS OF *PSEUDOCOCCUS BREVIPES*

These are described more fully in a concurrent paper.³ Since this study concerns only the rod-shape symbiont and its forms, no reference is made herein to the so-called "common symbiont." Previous data⁴ on the pres-

³ Carter, Walter. The symbionts of *Pseudococcus brevipes* (Ckl.). Ann. Entom. Soc. Amer. 28: 60-64. 1935.

⁴ See footnote 2.

ence of the organism in the mycetome of green-spotting mealy bugs have been fully confirmed by these later studies. Dissections of such mealy bugs have, without exception, shown the organism present in large numbers (Fig. 2, A). When a long series of mealy bugs from a green-spotting mealy-bug colony is taken and dissected without first individually testing for green spotting, the symbiont is found in varying numbers in different individuals, in some cases being recordable as present only in very small numbers. Colonies of the type listed in figure 2 show this variation in numbers of the

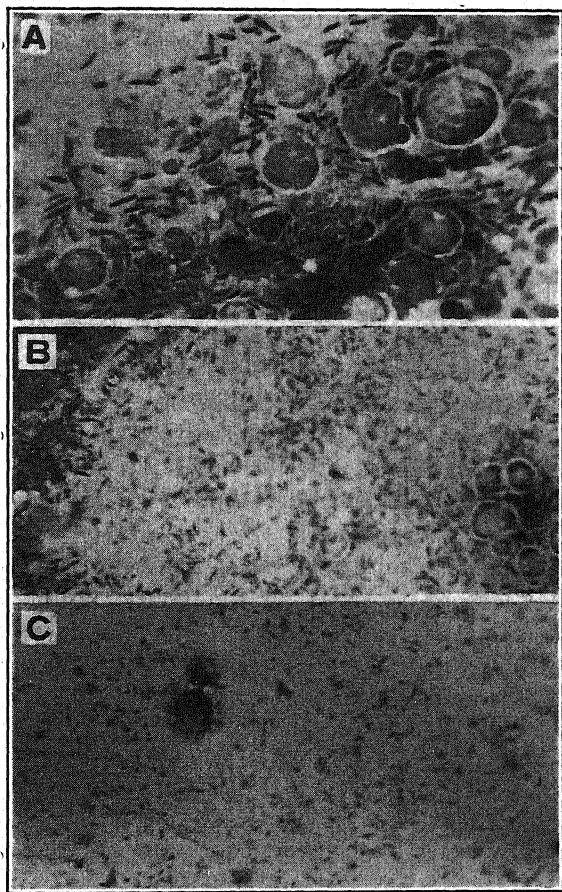


FIG. 2. The rod-shape symbiont of *Pseudococcus brevipes* (Ckl.) and its pleomorphic forms. A. Typical rod from green spotting mealy bug. B. Intermediate form. C. Typical coccus rod from non-green spotting mealy bug.

symbiont to a pronounced degree. The evidence of such series establishes the fact that the symbiont "culture" in the mycetome of the insect shows fluctuations in numbers common to other active populations of organisms.

EFFECT OF THE HOST PLANT TRANSFER

B. On the rod-shape symbiont. When green-spotting mealy bugs are transferred from pineapple to panicum grass, the organism disappears. This disappearance is coincident with the loss by the mealy bug of the capacity to produce green spots. In table 1 the presence or absence of the organism is shown. It is seen from this table that as long as progeny produced spots after being removed from the panicum grass to pineapple leaves, the symbiont could be found. When larger populations were used and only a small percentage of the individuals produced green spots, the organism was found only in those individuals that produced green spots. When transfer of mealy bugs back to pineapple leaves no longer resulted in the production of green spots, dissections showed the complete disappearance of the symbiont.

The optical equipment used to determine the presence of the organism was a 4-millimeter objective and a $\times 5$ ocular. With practice, a 16-millimeter objective could be used for preliminary viewing of preparations. An occasional case where the symbiont appeared to be smaller than normal led to the use of higher magnifications. It was then found that these occasional smears contained the organisms apparently in the process of dissolution. The best marked case of this kind encountered is shown in figure 2, B. In this preparation, normal symbionts, more faintly staining than the other forms present, were found, but the majority of the organisms present were narrower and shorter than the normal, while, with a 1.4 objective and a $\times 10$ ocular, extremely small bodies varying from a short rod to a coccus form could be seen. These last-named are shown in figure 2, C.

Evidence that the symbiont was pleomorphic was thus obtained and from that time search was made, first with the 4 millimeter $\times 10$ for the rod-shape form and then with the 1.4 millimeter $\times 10$ for the smaller form.

Reference to table 1 and figures 1 and 2 will show that the transition or intermediate form is found in rare cases in first- and second-generation mealy bugs on panicum, but that the normal form for mealy bugs from non-green-spotting colonies is the coccus rod. Table 2, which is a colony history of a mealy bug colony covering a much longer period of time, shows that the coccus rod is the stable form under the laboratory-greenhouse conditions in which the colonies are kept. Table 2 also shows that the coccus-rod populations vary in such mealy bugs, since in many preparations they are found only with great difficulty. The intermediate form is closer in form to the coccus-rod than to the rod-shape form, and occasionally preparations are found that contain scattered individuals in a coccus-rod population that are larger than the average coccus rod. Such cases are recorded as intermediates, though exact criteria for determination are not available. The 3 cases recorded in table 2, under date of January 5, 1934, are cases in point.

TABLE 2.—*Green spotting tests and dissections of mealy bugs after a green spotting colony had been transferred to panicum grass and had developed thereon from January 5, 1933, to August 12, 1933*

Dates	Green-spotting tests	Dissections				
		No.	Symbionts			
			Rod	Intermediate	Coccus rod	Absent
8/12/33	Negative	21	0	0	10	11
9/16/33	"	0				
10/31/33	"	0				
12/5/33	"	0				
1/5/34	"	14	0	3 ^a	14	0
2/13/34	"	0				
5/1/34	"	10	0	0	10	0

^a Determination based on a few scattered individuals in coccus-rod population that appeared slightly larger than average.

DISCUSSION

The symbionts of *Pseudococcus brevipes* are host-bound and are not themselves transmitted by the insect's feeding.² The relationship between the rod-shape symbiont and green spotting must therefore be brought about by the influence of the former on the oral secretions of the insect. In the case of *P. brevipes*, the mycetome is invariably found in close apposition with the large mid-gut of the insect, although no connecting duct between the two has been observed.

The factor of time involved in the complete disappearance of the symbiont from the insect's mycetome suggests that the critical time is when the transfer of the symbionts takes place from the mother to the young. Indications of this are the persistence of green spotting in mealy bugs that have fed on panicum for short periods of time only (10–14 days), the partial disappearance when the mealy bugs are first applied to panicum as unfed crawlers, and the complete disappearance of the organism in mealy bugs whose parents have lived their entire lives on panicum.

Without recognition of the transitional or intermediate form of the symbiont, the relationship between the rod-shape form and the coccus-rod form could not have been suspected. In addition to the cases tabulated, the intermediate form together with sparse numbers of the rod-shape form, has been found in individuals from a green-spotting colony which were temporarily unable to produce green spots. This suggests that fluctuations in the rod-shape symbiont population in individuals of a green spotting colony are responsible for the proportion of these individuals which at any one time are unable to produce green spots on the first leaf to which they are transferred.

With the normal development of the colony, such individuals or their progeny become capable of producing green spots through the development of the rod-shape population in the insect. When, however, the food plant of the insect is radically changed, as it is when panicum grass is substituted for pineapple, the life cycle changes and, instead of the organism merely fluctuating in numbers, or from rod to intermediate stage and back to rod, there is a degeneration to the minute coccus-rod form. Populations of this last form are always relatively low compared with those of the rod and frequently are so small as to make determination difficult.

The field situation over most of the pineapple-growing area of Hawaii is one wherein the green-spotting capacity, in greater or less degree, is always present and, under natural conditions, it is evident that the stability of the coccus-rod condition is broken in some way and the rod-shape symbiont, with its concomitant green-spotting capacity, restored. In the laboratory-greenhouse conditions, wherein all the colonies are strictly isolated, this has not yet taken place, even though mealy bug colonies from which the green-spotting capacity has been eliminated by feeding on panicum grass, have been fed on pineapple plants for long periods of time.

While the restoration of the rod-shape form and the green-spotting capacity would be a significant completion of a chain of evidence, especially if the stimulus could be determined, the facts indicate that the mere change of food was not responsible for the loss of the mealy bug's capacity to produce green spots. If the latter were true, then the colony should promptly resume its green spotting when returned to pineapple. On the other hand, the invariable presence of the rod-shape form in green-spotting mealy bugs, the evidence of a transitional form, the complete absence of the rod and the invariable presence of the coccus-rod in non-green spotting mealy bugs are considered to be proof of the relationship between the symbionts and oral secretions of the insect.

SUMMARY

The relationship between the symbionts of *Pseudococcus brevipes* (Ckl.) and the phytotoxic secretion of the insect that is responsible for green spotting of pineapple leaves has been studied. Green-spotting mealy-bug colonies were transferred from pineapple to *Panicum barbinode* Trin. and transferred back to pineapple after varying lengths of time.

The effect of the colonies' feeding on the grass was to eliminate the green-spotting capacity of the mealy bugs; continued growing of these colonies on pineapple has failed to restore this capacity.

Coincident with the loss of the green-spotting capacity is the disappearance of the rod-like symbiont from the mycetome of the insect. The evidence is that this symbiont is pleomorphic and passes from a rod-shape to a coccus-rod form under the influence of radically changed nutrition.

The rod-shape form is invariably present in green-spotting mealy bugs; the coccus-rod in non-green spotting mealy bugs. The fact that return of the mealy bugs to pineapple does not result in the return of the capacity to produce green spots is proof that the loss of this capacity in the first instance is not due merely to a changed food plant, but to a radically changed physiology that is clearly associated with pleomorphism of the insect's symbionts.

PINEAPPLE EXPERIMENT STATION,
P. O. Box 3166,
HONOLULU, HAWAII.

SCLEROTINIA ROT OF SQUASH AND PUMPKIN¹

PAUL A. YOUNG

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Many rotting spots developed in squash and pumpkin fruits in gardens and in storage in Bozeman, Montana, in 1933 and 1934. These fruits were studied to determine the cause of the disease.

REVIEW OF LITERATURE

Young and Morris (23) and Young (22) described the sclerotinia stem canker of sunflower and hollyhock. Joshi (10) described sclerotinia wilt of safflower, and reported that artificial inoculations with this fungus caused infections in wheat, oats, and gram. The correct name of the causal fungus is *Sclerotinia sclerotiorum* (Lib.) deBary, as determined by Brooks (4). Young (21) gave a preliminary description of sclerotinia rot of pumpkin and squash.

This sclerotinia continues to attack an increasingly large number of individuals and species of economic plants, and to infest increasing amounts of the best agricultural soil. This fungus does its worst economic damage to cultivated species mostly in the following families: *Compositae*, *Cruciferae*, *Cucurbitaceae*, *Chenopodiaceae*, *Leguminosae*, *Solanaceae*, and *Umbelliferae*. It probably will become more destructive in the countries with temperate climates. Consequently, the following list of hosts with citations is given for the convenience of the many people who study and work to control this extremely destructive fungus.

Plants diseased by *Sclerotinia sclerotiorum*

Aegopodium (14)	Cabbage (3, 6, 18)	Chrysanthemum ^{a,b}
Alfalfa (2, 3)	Campanula (7, 23)	Clover (2, 18)
Amaranthus ^a (23)	Canada thistle ^a (3, 23)	Cnicus (3)
Angelica (15)	Cantaloupe (1)	Columbine (17)
Antirrhinum (3, 7)	Caraway (6)	Cucumber (6, 19)
Apple (3)	Cardamine (3)	Dahlia (17, 23)
Apricot (16)	Carrot ^a (3, 6, 18)	Delphinium (3, 15)
Aquilegia (1)	Cauliflower (6, 18)	Easter lily (1)
Argemone (10)	Celery ^a (6)	Eggplant (6)
Artichoke (4, 18)	Cerefolium (14)	Fanweed ^a (23)
Asphodelus ^c (10)	Cheiranthus (3, 7)	Fennel (18)
Bean ^a (6, 19, 23)	Chenopodium ^a (23)	Fig (20)
Beet ^a (6, 18, 23)	Chicory (6, 18)	Flax (12)
Brassica ^a (3, 15, 23)	China aster (15)	Foeniculum (14)

¹ Contribution from Montana State College, Agricultural Experiment Station, Paper 51, Journal Series.

Forsythia (15, 18)	Mustard (3, 18)	Schizanthus (7)
French endive ^a (23)	Myosotis (11)	Silybum (3)
Ginseng (17)	Oat ^c (10)	Sonchus (3, 23)
Gram ^c (10)	Omphalodes (18)	Sour orange (3, 15)
Hemp ^a (15, 18)	Onion (6)	Soybean (2, 3)
Hollyhock ^a (22)	Parsley (6)	Spinach (6)
Ipomoea (15)	Parsnip (2, 3)	Squash ^a (21)
Iris (1)	Passiflora (3)	Strawberry (1)
Iva ^a (23)	Pea ^a (3, 6, 23)	Sugar beet ^{a, c} (23)
Jerusalem artichoke (9)	Pear (3)	Sunflower ^a (23)
Kale (6)	Pepper (6)	Swede (3)
Kohlrabi (6)	Petunia (14)	Tobacco (1, 3)
Lamium (18)	Potato ^a (3, 6, 23)	Tomato (3, 6, 18)
Lathyrus (19)	Pumpkin ^a (3, 21)	Trigonella (13)
Lavatera (3)	Radish (14, 18)	Turnip (3, 6)
Lemon (3, 18)	Rape (3, 18)	Vicia (3, 15, 18)
Lettuce ^a (6, 18)	Rhubarb (6)	Violet (19)
Lupinus (1, 3)	Rock melon (3)	Watermelon (1, 3)
Mangel-wurzel (6)	Rutabaga ^a (6, 23)	Wheat ^c (10)
Melilotus ^{a, b}	Safflower (10)	Zinnia ^a (3, 14, 18)
Mulberry (5, 18)	Salsify (15)	

^a In Montana.

^b Described in this article.

^c Artificial inoculation.

MATERIALS AND METHODS

Rotting fruits of pumpkin and squash were brought to the laboratory and a fungus from them was isolated on agar. The agar cultures were inoculated into shallow wounds in healthy fruits of squash and pumpkin to determine under laboratory conditions the symptoms of infection. The fungus was reisolated from the artificially infected fruits.

Sclerotia produced in naturally infected pumpkins and squashes were planted in sand in flower pots in the greenhouse. The sand was covered with cloth and kept wet during a few months to facilitate production of apothecia by the sclerotia. Henson (8), Joshi (10) and Young (22) produced apothecia from sclerotia on agar.

EXPERIMENTAL RESULTS

In the pumpkins (*Cucurbita pepo* L.), the mold caused a rapidly developing soft rot that released a large amount of liquid, and produced dense masses of white mycelium with numerous black sclerotia ranging in size from 0.5 to 15.5 cm. long (Fig. 1, A, D). Large masses of mycelium grew from the sides of pumpkins set with their infected spots over jars (Fig. 1, C).

The mold caused disc-shape, rapidly enlarging, water-soaked spots 1 to 10 cm. wide in the squash (*Cucurbita maxima* Dach.) fruits (Fig. 1, B). These spots developed a dry rot that changed Buttercup and Table Queen

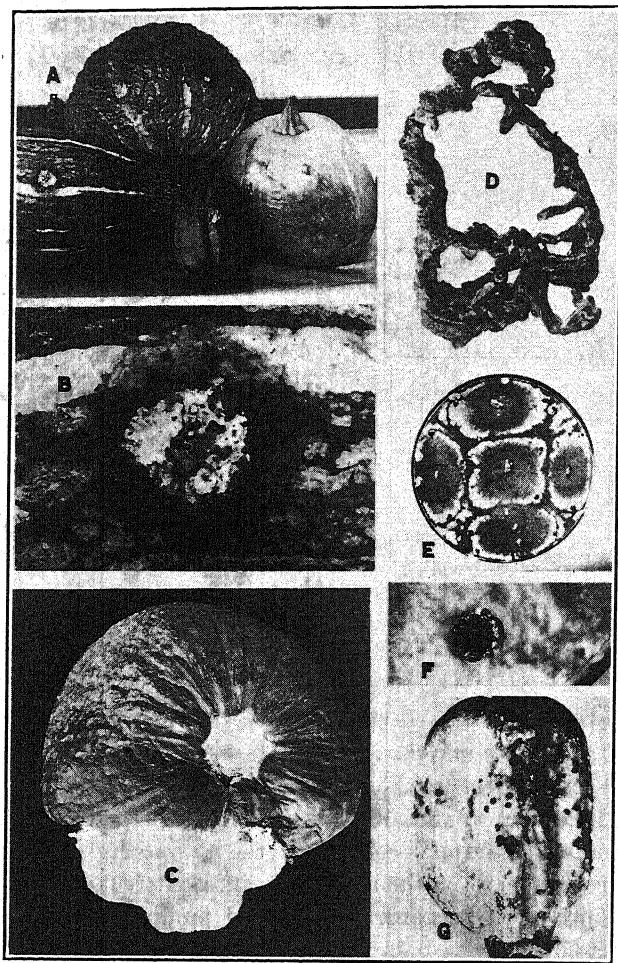


FIG. 1. *Sclerotinia sclerotiorum*. A. Epidermal wrinkles in a Winter Luxury pumpkin, and fruit spots with white mycelium in Winnebago, Warrens Improved, and Table Queen squashes, as symptoms of natural field infection with *Sclerotinia*. The infected parts did not touch the soil. The globular Warrens Improved squash weighed 21 lb. B. Large water-soaked ring surrounding *Sclerotinia* hyphae in a Winnebago squash. This ring marked tissues killed by the fungus. C. Mass of *Sclerotinia* mycelium produced on a Small Sugar pumpkin. $\times 4/17$. D. A sclerotium of *Sclerotinia* produced inside a naturally infected Small Sugar pumpkin. $\times \frac{1}{2}$. E. Antagonism for each other shown by 5 separate colonies of *S. sclerotiorum* in a pure culture on an agar plate. This fungus was reisolated from an artificially inoculated Buttercup squash. $\times \frac{1}{2}$. F. Juvenile sclerotium with exuded liquid droplets. *Sclerotinia* isolated on agar from a Winter Luxury pumpkin. $\times 1$. G. Artificially inoculated Table Queen squash bearing sclerotia and mycelium of *Sclerotinia*. Many of the sclerotia were like that in F. Squashes rotting in storage bore almost no external mycelium.

squashes into dry mummies containing many sclerotia. The seeds were decomposed in the rotted squash and pumpkin fruits.

In 1933, most of the squash and pumpkin fruits were infected in spots that did not touch the soil. The infections appeared as small, water-soaked spots with white mycelium protruding from their centers (Fig. 1, A, B). Droplets of yellow liquid were exuded from the margins of many of the infected spots. The infection of these fruits was associated with 2.72 inches of rain from Aug. 21 to 27, 1933. These fruits exude sap from wounds, and this sap would serve as a culture medium for the saprophytic development of ascospores of *Sclerotinia* preceding infection. Hence, the infection of the tops of these fruits may have been caused by ascospores of *Sclerotinia* discharged during the rainy period. During 1934, the fungus entered the bottoms of the fruits touching the soil, so mycelium in the soil presumably caused these infections.

Isolations from the rotting pumpkins and squashes yielded many pure cultures of *Sclerotinia sclerotiorum*. The mycelium on both agar and fruits aggregated to form dense masses that exuded many droplets of liquid, while they produced black, hard sclerotia (Fig. 1, D, F).

The *Sclerotinia* on agar was placed in wounds in 12 healthy fruits of pumpkin and squash in the laboratory. Ten of the fruits developed typical sclerotinia rot from the wound inoculations (Fig. 1, G). The 27 fragments of host tissues taken from these fruits and placed on agar all made pure cultures of *Sclerotinia sclerotiorum* (Fig. 1, E).

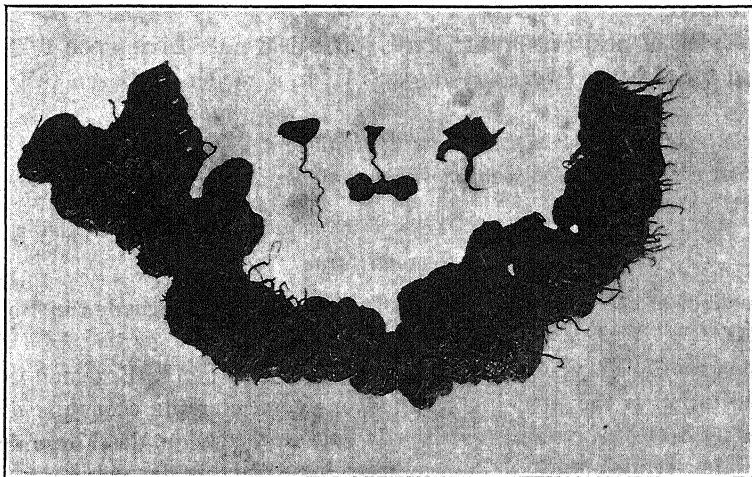


FIG. 2. Apothecia of *Sclerotinia sclerotiorum* produced by sclerotia planted in sand. These sclerotia had grown in pumpkins. The central mature apothecium is attached to its sclerotium. The large sclerotium bore only immature apothecia. $\times 1$.

During the first 2 weeks of growth, none of the 5 main colonies coalesced in one of these plates of *Sclerotinia sclerotiorum* (Fig. 1, E). This antagonism between the colonies was less definite than the antagonism previously observed between colonies of other fungi and bacteria.

Many sclerotia from naturally infected squash and pumpkin fruits were planted in sand in the greenhouse. The sclerotia from squash did not produce apothecia. However, the sclerotia from two pumpkins produced 17 typical apothecia and many filiform, atypic apothecia within 128 to 208 days (Fig. 2).

Besides the hosts previously reported by Young and Morris (23) and Young (21), *Sclerotinia sclerotiorum* naturally infected and caused serious diseases in the following economic plants in Montana.

Compositae

Root and stem rot of shasta daisy, *Chrysanthemum maximum* Ram., at Ronan in 1933.

Drop of head lettuce, *Lactuca sativa* L., in a field near Bozeman in 1929.

Stem rot of *Zinnia elegans* Jacq. in a garden in Bozeman in 1934.

Leguminosae

Stem rot of yellow sweet clover, *Melilotus officinalis* Lam., in a greenhouse at Bozeman in 1932.

Stem rot of white sweet clover, *Melilotus alba* Desr., in a field at Bozeman in 1930.

Stem rot of pea, *Pisum sativum* L., in a field near Livingston in 1932.

Pod rot of bean, *Phaseolus vulgaris* L., in a garden in Bozeman in 1933.

Solanaceae

Stem rot of potato, *Solanum tuberosum* L., in a greenhouse in Bozeman in 1931.

Umbelliferae

Root rot of carrot, *Daucus carota* L., *sativa* DC., in a garden in Bozeman in 1929.

Stem rot of celery, *Apium graveolens* L., in a garden in Bozeman in 1930.

Sanitation is the principal method of decreasing the economic damage caused by *Sclerotinia sclerotiorum*. It lives in the soil on dead organic matter besides living plants, so destroying diseased plants decreases the spread of the fungus in the soil. Soil sterilization controls sclerotinia in greenhouses. Rotation of crops and the use of resistant plants are practical methods of control.

SUMMARY

Sclerotinia sclerotiorum caused a wet rot of pumpkin fruits and a dry rot of squash fruits, producing abundant white mycelium and many large black sclerotia in the fruits. In pumpkin, it produced sclerotia 0.5 to 15.5 cm. long. These sclerotia produced apothecia when planted in sand.

This *Sclerotinia* was isolated from naturally infected squashes and pumpkins; was inoculated into healthy squashes and pumpkins in which it caused typical rot; and was reisolated from these artificially inoculated and rotting fruits.

The following are reported as new hosts of this *Sclerotinia* in Montana: Bean, carrot, celery, lettuce, pea, potato, shasta daisy, white sweet clover, yellow sweet clover, and zinnia.

The known hosts of *Sclerotinia sclerotiorum* are listed, with citations.

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INOCULATION OF RABBITS WITH *ELSINOE AMPELINA*

ANNA E. JENKINS AND LEIGH T. GILTNER

(Accepted for publication February 2, 1935)

Charrin and Le Play's report of the infection of animals (only rabbits are named) with a culture of *Elsinoe ampelina* (de Bary) Shear,¹ or what they supposed to be this fungus, was published by Viala and Pacottet (7, p. 521-523) in connection with their discussion of anthracnose of grape (*Vitis* sp.), which is caused by this organism. The writers' recent inoculations of rabbits with an authentic culture of this plant pathogen gave only negative results. These experiments are here described and certain other sustaining data presented, particularly because they explain to some extent Charrin and Le Play's results as interpreted by them, thus serving as a correction of the literature as it stands at present.

In Charrin and Le Play's report of their inoculations, supposedly with *Elsinoe ampelina*, reference is made to their previous infection (3) of animals with another fungus from grape, *Stearophora radicola* Mangin and Viala (4). Severe infection was obtained with both rabbits (3, 4) and guinea pigs (4). *Oospora guignardi* also is mentioned by Charrin and Le Play (7, p. 523) as associated with lesions of a rabbit; it is believed, however, that the intended reference is to an undetermined species of *Oospora* isolated by Charrin and Delmare (2) from a cystic tumor of a rabbit and identified (2) as closely related to this grape fungus. The cultures of the *Stearophora* were provided by Viala and Pacottet and the inoculations were made by means of subcutaneous and intraperitoneal injections of the fungus (3). It is inferred that the cultures, supposedly of *E. ampelina*, were obtained also from Viala and Pacottet and that the inoculations with it were made by means of intraperitoneal, if not also subcutaneous, injections of the fungus.

In each of the 2 inoculation experiments performed by the writers 3 groups of rabbits were inoculated. Of these groups, consisting of 2 animals each, the first received subcutaneous inoculation, the second, intraperitoneal, and the third, intravenous. The culture of *Elsinoe* employed as inoculum was isolated in October, 1924, from anthracnose lesions on grape from Florida, and it had produced severe infection when inoculated on grape.² Cultures grown on potato-dextrose agar slants were employed as in the case of the inoculation experiments on the plant suspect. In the first experiment the cultures were 3 months old and in the second, 1 month old (Fig. 1, A).

¹ As discussed by Shear (5), this fungus originally described as *Sphaeloma ampelinum* de Bary (1) was assigned the name *Manginia ampelina* by Viala and Pacottet (6, p. 145-150).

² Unpublished data.

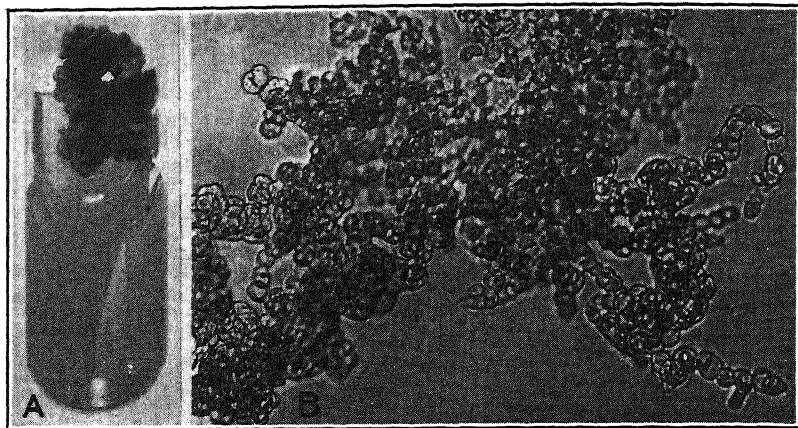


FIG. 1. *Elsinoe ampelina*. A. Month-old growth of the fungus on potato-dextrose agar. B. Young hyphae from a plating on beef agar. $\times 260$.

In each case the purity and vitality of the cultures were determined by making platings from them on beef agar several days before the inoculations were to be made. Hyphal growth composing young cultures from a plating is shown in figure 1, B. By platings similar to those just described, it was demonstrated also that the growth of the organism was not inhibited by the saline solution.

Although they may have been present in some instances, no conidia were found in the mounts made from the potato-dextrose agar cultures actually used for inoculating grape, or rabbit, nor in the beef-agar cultures. They form readily, however, when cultural growth is transferred to a moist atmosphere; if conidia are necessary for infection of grape, in the inoculation experiments performed they must have formed soon after the water infusion of the culture was applied to the plant.

That the results of the inoculation experiments on rabbits would be negative was rather to be expected, if for no other reason than that the *Elsinoe* would probably not be viable at its body temperature, *i.e.*, 38° – 39° C. In lieu of information on the exact thermal death point of the fungus, cultures were incubated at $37\frac{1}{2}^{\circ}$ C., which is slightly lower than the body temperature of the rabbit.

The first inoculation experiment was performed on June 27. One of the subcutaneously inoculated animals became injured in the cage on July 20, later developing posterior paralysis. It died on August 6. The autopsy revealed a small local abscess at the site of inoculation; but no gross lesions attributable to the *Elsinoe* were found. The 5 remaining animals included in the experiment were autopsied on August 7 and 9. On the other subcutaneously inoculated animal there was also a small abscess at the site of

inoculation. . . A small pedunculated nodule containing dry, yellowish pus and probably of parasitic origin was found in the peritoneum of one of the animals inoculated intraperitoneally. No gross lesions attributable to the inoculated organism were present, however, and the animals were well nourished.

In the second inoculation experiment, performed on August 3, one of the animals died on September 12. The autopsy here revealed the presence of an enteritis, which was probably largely responsible for the death of the rabbit. No lesions attributable to the *Elsinoe* were found. The other 5 animals included in this experiment were autopsied on October 15 and 16. Small local abscesses were again present at the site of inoculation of the 2 animals subcutaneously inoculated; otherwise, no lesions attributable to the *Elsinoe* were found. The animals were in a state of good nutrition.

It is believed that in both experiments the local abscesses resulted merely from the introduction of a foreign substance under the skin.

At the time of autopsy cultures were made from the heart's blood, and internal organs, lungs, liver, kidneys and spleen, of all of the inoculated animals, with the exception of the one that died on August 6, whose internal organs were not suitable for culturing; the four local abscesses and the peritoneal lesion also were cultured. The media used were potato-dextrose, glycerin, and Sabouraud's maltose agar, all of which are favorable for the growth of the *Elsinoe*. In no case was the fungus isolated and for the most part the cultures remained sterile. Some developed *Bacillus coli* and a few showed fungus growths.

The several sets of cultures incubated at 37.5° C. for approximately 10 days ceased their development at this temperature and failed to grow when removed to room temperature, or when transferred to fresh media. The fact that the fungus was killed at 37.5° C. not only substantiates the negative results of the inoculation experiments here reported, but it also precludes the possibility of the infection of rabbits by this fungus as reported by Charrin and Le Play.

A partial solution, at least, of the positive results reported by Charrin and Le Play, i.e., if the lesions they describe were caused by the inoculum they used, is that this was entirely of some other fungus, or was impure for the *Elsinoe*. If their cultures were obtained from Viala and Pacottet, as has already been suggested, they may well have been mostly or entirely of some other fungus. In support of this statement may be cited Viala and Pacottet's assertion that the fungus they studied as *Elsinoe ampelina* was similar to *Colletotrichum lindemuthianum* in general cultural appearance (8, p. 375) and that it was of rapid growth in culture (6, p. 120-121). Neither characteristic applies to the *Elsinoe*. This fungus grows so slowly in culture that a faster growing organism associated with it on anthracnose lesions

could have been isolated much more readily than the *Elsinoe*, and then have been confused with it. On other grounds Shear (5) has previously questioned the purity of Viala and Pacottet's cultures, supposedly of *E. ampelina*.

BUREAU OF PLANT INDUSTRY
AND BUREAU OF ANIMAL INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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AUSTRALIAN CITRUS SCAB CAUSED BY SPHACELOMA FAWCETTII SCABIOSA

ANNA E. JENKINS

(Accepted for publication May 29, 1935)

The "yellow rough scab" (1) of lemon leaves, said to have been common in Queensland in 1876, evidently is the same as the "orange leaf scab" that Tryon (1) reported from Toowoomba, Queensland, in 1889, attributing it to *Ramularia* sp. Ten years later McAlpine (2) designated it as *R. scabiosa* McAlpine and Tryon. He used the term "scabbing" of citrus in a collective sense; and, referring to the Queensland disease reported in 1876, suggested that it might be traced to "some of the native Citrus trees." Florida citrus scab or verrucosis, first attributed to *Cladosporium* sp., now identified as *Sphaceloma fawcettii* Jenkins, he said had not yet been detected in Australia.

At the request of E. J. Butler, 2 specimens of scab collected by Tryon at Toowoomba, and doubtless sent by him to McAlpine and retained at the Department of Agriculture, Melbourne, Victoria, were transmitted to the writer by D. B. Adam (Dec. 3, 1926). Both are labelled in what is probably McAlpine's handwriting, one (Fig. 1, G) as *Ramularia scabiosa*, the other (Fig. 1, H and I), described as occurring on lesions with the *Ramularia*, as *Phyllosticta scabiosa* McAlpine. The 2 specimens, both showing the *Ramularia*, are then of authentic value, and are probably to be classed as type specimens. The *Ramularia* on this material is certainly the same as the fungus on a similar specimen of what is now called scab in New South Wales, kindly contributed (May 12, 1926) by G. P. Darnell-Smith. As the author wrote to Dr. Darnell-Smith (Aug. 30, 1926), this is a *Sphaceloma* similar to *S. fawcettii*, but sufficiently distinct to suggest a different species. The conidia (often $10-17\ \mu \times 2.5-5\ \mu$) and conidiophores are noticeably larger than in *S. fawcettii*. The lesions are also larger, as well as more regularly discoid or crateriform than those of the Florida citrus scab, and the more robust fructifications are more easily seen (Fig. 1, A-F). These are grayish, hair brown or fuscous (3). Fructifications of *S. fawcettii* are ordinarily more delicate in appearance and generally drab, although they may become dark. The difference in the lesions may be seen by comparing illustrations of the 2 diseases found in the literature (4, 5). For the present time at least these considerations seem to necessitate making a new name for *R. scabiosa*, and for this *Sphaceloma fawcettii scabiosa* is proposed.

McAlpine reported citrus scab caused by the form of *Ramularia scabiosa* on leaves only, although Tryon also recorded it on fruit. The fruit scabbing that McAlpine attributed to several new species of imperfect fungi, including *Cladosporium subfusoideum*, also recorded on leaves, is evi-

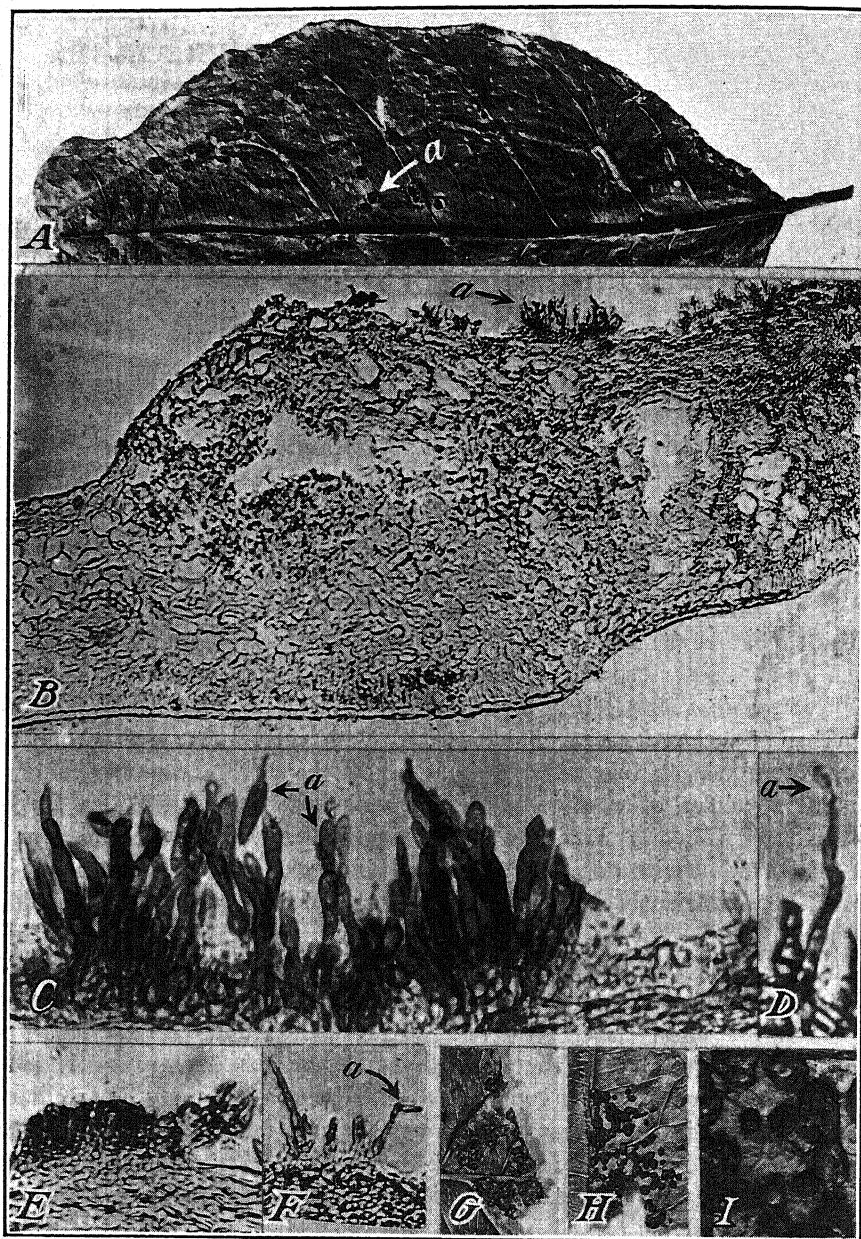


FIG. 1. A-F. *Sphaceloma fawcettii scabiosa* on lemon leaf from G. P. Darnell-Smith, N. S. Wales. A. Portion of leaf. $\times 1$. B. Section of leaf through lesion at A, a. $\times 100$. C, D. Conidiophores bearing conidia (C, a and D, a), those in C enlarged from B, a. $\times 600$. E. Acervulus at edge of lesion. $\times 400$. F. Additional conidiophores and a conidium (F, a). $\times 400$. G-I. *Ramularia scabiosa*, original collections by Trvon.

dently the same disease. The late N. A. Cobb, who worked many years in Australia, agreed with the writer that the grey scab of lemon he reported (6) in New South Wales was apparently Australian citrus scab. It is probable that the scab of citrus first reported in New Zealand by Kirk (7), as well as the disease known in that country as grey scab (4) of citrus, may be the same disease.

The early Queensland scab record (1876) constitutes the first definite report of a disease of citrus caused by *Sphaceloma* in any country, as well as one of the first *Sphaceloma* diseases ever reported. Further field and herbarium taxonomic study should be made to determine whether *Sphaceloma fawcettii scabiosa* should properly be given specific rank and whether, as suggested by McAlpine, it is perhaps indigenous to the Australian continent on native citrus.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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PHYTOPATHOLOGICAL NOTE

Soil Nematodes in Forest Nurseries.—During the last 2 years there have been observed in Wisconsin forest nurseries several cases of a direct correlation between the severity of damping off of coniferous seedlings and the number of microscopic eel worms, or nematodes¹ occurring in soil. It was particularly striking that the parasitic organisms in the soils heavily infested with nematodes were highly resistant to the common methods of damping-off control. In all cases the soils had been previously treated with farm manure. All of the nematodes observed possessed the same common characteristics; they were smaller than 0.3 mm. in length and .02 mm. in width, with a cylindrical, elongated, transparent, nonsegmented body, pointed at both ends (Fig. 1). They compared closely with the genus *Rhabditis*, but no attempt was made to identify the species.

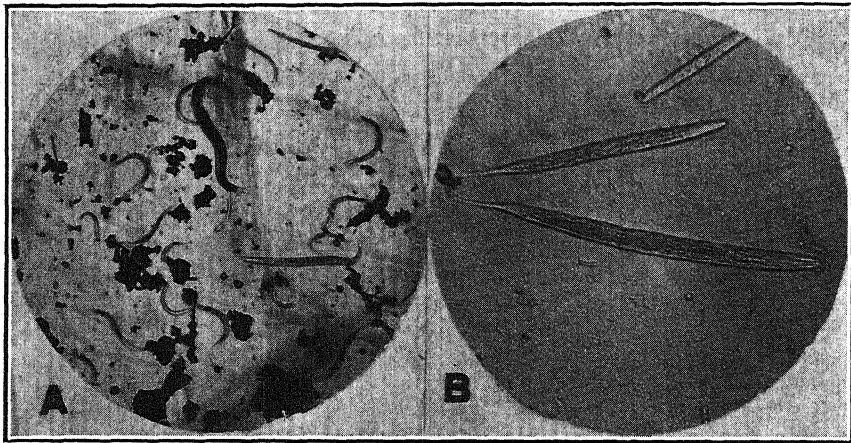


FIG. 1. A. Living nematodes, larvae and adult. $\times 85$. B. Dead larvae of *Rhabditis* sp. $\times 210$.

At first, it was believed that the nematodes promoted a more rapid infection with disease or protected the consumed spores of the pathogenic fungi against the fungicides of insufficiently strong concentration. Later on, however, the nematodes were found in great number in the tissue of live seedlings shortly after germination. This observation furnished strong evidence that the organisms in question acted as primary parasites, although

¹ For general description of nematodes see: S. A. Waksman, *Principles of Soil Microbiology*, 1932, 2d ed., Baltimore, p. 326-335 and Marcinowski, K. *Parasitisch u. semi-parasitisch an Pflanzen lebende Nematoden*. *Arb. K. Biol. Anst. Land u. Forst.* 7: 1-192. 1909.

no reinoculation experiments were carried on because of the technical difficulties involved in the isolation of nematodes in pure culture.

The microscopic observations showed that the nematodes are highly resistant to fungicides. Instant death of larvae took place only when they were brought in contact with 2 per cent by volume of H_2SO_4 solution. The animals survived for 5 minutes in 1 per cent H_2SO_4 and in 2 per cent HNO_3 , and for more than 15 minutes in 2 per cent solution of formaldehyde and 7 per cent solution of aluminum sulphate.

Pot experiments in the greenhouse with the infested soils, using different fungicides and a variety of coniferous species, have shown that the control of parasites in the nurseries will not be possible in practice with the use of any chemical except 2 per cent sulphuric acid (2 parts of conc. com. acid, sp. grav. 1.8 to 100 parts of water). The latter gave satisfactory results when applied in sufficient quantity to saturate the soil in the entire jar and when the excess was removed by watering. This treatment on granitic sand nearly doubled the amount of available phosphorus, but removed the available nitrogen and most of the available potash. An application of organic matter and mineral fertilizers was absolutely necessary for the proper growth of the seedlings. The common practice of sprinkling on the soil an equivalent amount of acid in a high concentration (about 1 to 7) produced inconsistent and unsatisfactory results.

Although the experiences and observations described are of a purely incidental character, they suggest that some species of nematodes, either directly or indirectly, participate in the destruction of coniferous seedlings during the early period of growth. The writer believes, therefore, that proper attention to these organisms may prevent the sad experience of the citrus growers, which is briefly reported by N. A. Cobb² as follows: "Our ignorance concerning nematodes in general, and soil-inhabiting nematodes in particular, is well illustrated by the history of the citrus-root parasite *Tylenchus semipenetrans*, which within few months of its discovery in California has been located in such widely separated places as Florida, Spain, Malta, Palestine, and Australia. This series of events is, the writer believes, simply illustrative of the surprises in store whenever the soil-inhabiting nematodes receive at the hands of agricultural scientists the attention they merit."—S. A. WILDE, University of Wisconsin.

² Citrus-root nematode. Jour. Agr. Res. 2: p. 217. 1914.

The American Phytopathological Society, in business session December 31, 1935, unanimously approved the action of the Council levying a charge of \$1.00 per printed page of all contributions accepted for publication in PHYTOPATHOLOGY on and after January 1, 1936, in accordance with the authority granted at the Atlantic City meeting in December, 1932.

It was voted also to charge to the author the cost of all illustrations in excess of that of 2 full-page halftones, or equivalent, accompanying any one paper. Payment should be made by check or money order to H. A. Edson, Treasurer, Bureau of Plant Industry, Washington, D. C.

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IMMUNOLOGICAL STUDIES ON THE THREE PEACH DISEASES, YELLOW, ROSETTE, AND LITTLE PEACH¹

L. O. KUNKEL

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INTRODUCTION

It has been shown that plants of *Nicotiana sylvestris* Spegaz. and Comes affected by ordinary tobacco mosaic or by mild strains of this disease become immune from aucuba mosaic of tomato, which is caused by a severe strain of the tobacco-mosaic virus (4). Similar plants affected by other virus diseases, such as tobacco ring spot or cucumber mosaic, are not protected against aucuba mosaic. It likewise has been shown that zinnia plants affected by ordinary cucumber mosaic or by mild strains of this disease are immune from infection by a severe strain of cucumber-mosaic virus, but that similar plants having other virus diseases such as tobacco ring spot or tobacco mosaic are not protected against the severe strain of cucumber mosaic (5). These immunity reactions are specific, and it is, therefore, possible by inoculations to appropriate test plants to determine quickly and accurately whether any new disease belongs to either the tobacco-mosaic or the cucumber-mosaic group. If immunity reactions are equally specific for other virus diseases of plants, they should be useful in detecting relationships between these maladies.

Three virus diseases of peach, yellows, rosette, and little peach, are prevalent in the eastern United States. Yellows, which was first observed near Philadelphia in 1791, spread gradually northward through the New England states and into Canada. It spread southward into Delaware, Maryland, Virginia, West Virginia, and North Carolina. Little peach and rosette were discovered about 100 years after yellows began to attract attention. Little peach has much the same geographical range as yellows. Rosette is somewhat more southern in its distribution than either of the other diseases. However, it occurs apparently as far north as Michigan (1). The three diseases are alike in certain respects. They all cause stunting,

¹ Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

the abnormal production of secondary shoots, and yellowing of mature leaves. None of them can be transmitted mechanically except by tissue transplantation. Yellows is more severe than little peach, and rosette more severe than yellows. It was thought that the three diseases might be caused by different strains of the same virus, and that evidence of such a relationship might be obtained from cross-immunity tests similar to those already described for the different strains of tobacco- and cucumber-mosaic diseases. Such tests have been made, and it is the purpose of this paper to report the results.

MATERIALS AND METHODS

Potted seedling peach trees grown in greenhouses from seed obtained from a commercial dealer at Germantown, Pennsylvania, were used in all experiments. Most of the trees were uniform in appearance. Any that showed unusual individual variations were discarded. No trees were found that proved resistant to any one of the three diseases. Except for slight color variations, the symptoms of each disease were essentially identical in different seedlings. All trees were held in unheated cold frames during a portion of each winter in order that they might undergo the necessary period of dormancy. Noninoculated control trees were provided in all experiments. They were not necessary as a check against accidental infections, for such infections do not occur in greenhouse-grown seedlings. The controls were found to be useful in making comparisons between diseased and healthy trees. The yellows virus was obtained from Dr. T. F. Manns, of the Delaware Agricultural Experiment Station, the rosette virus from County Agent Mr. W. C. Johnstone, Paducah, Kentucky, and the little-peach virus from a commercial orchard near Clinton, New Jersey. Inoculations were by buds transplanted to the stems of the trees and, since location of point of inoculation proved to be important, a record was kept of the approximate height at which the buds were inserted. Practically all transplanted buds lived, and all that lived transmitted disease. Buds from yellows trees, from little-peach trees, and from rosette trees are designated as "yellows buds," "little-peach buds," and "rosette buds," respectively.

SYMPTOMS

The symptoms of each of the three diseases are so well known and so adequately treated in the literature that it will not be necessary to describe them at length here. Since, however, there is evidence that distinct strains of at least two of the diseases, little peach and yellows, are prevalent in nature, and since certain symptoms either have not been mentioned previously or have not been emphasized sufficiently, a brief description of the diseases used in these experiments will be given. All of the symptoms to be described appeared in young seedlings growing under greenhouse conditions.

Little Peach

The first visible symptom in trees to which little peach has been transmitted is distortion of young leaves at the tips of affected branches. The surfaces of these leaves are rugose at this stage in their development. As the affected leaves approach maturity, their surfaces may become as smooth as those of leaves on healthy trees. Another early symptom, but one that does not appear in all trees, is the production of an abnormally large number of short branches along the main stem, especially near the soil level. These branches show a more upright habit of growth than do branches from

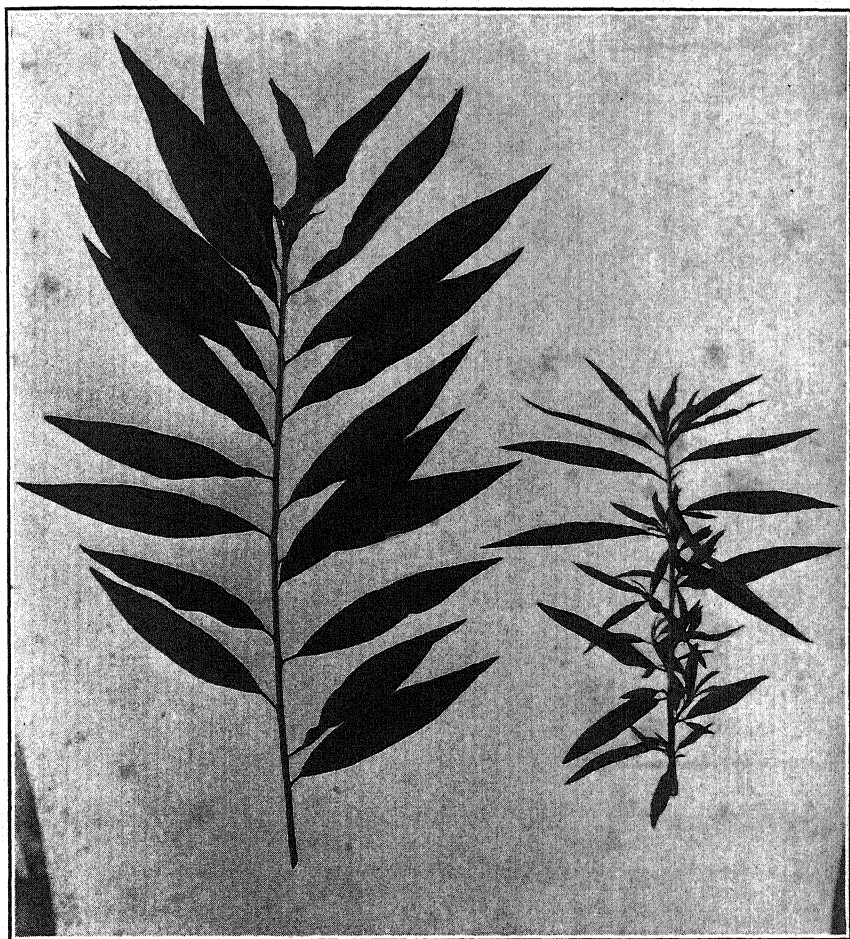


FIG. 1. Two branches from peach trees of the same age. The branch on the right shows the twiggy type of growth characteristic of little-peach disease; the branch on the left is healthy.

healthy stems, but they do not take a position so near the vertical as is characteristic of similar branches on yellows trees. The disease causes shortening of internodes and general stunting of trees. Leaves and young branches on newly-infected trees are usually a somewhat deeper green color than is normal for healthy trees. After the disease has become chronic, mature leaves turn slightly yellow and a twiggy growth, such as is shown in figure 1, develops along the branches. Trees that have been diseased for 2 or 3 years produce an abnormally small number of new shoots following a period of dormancy.

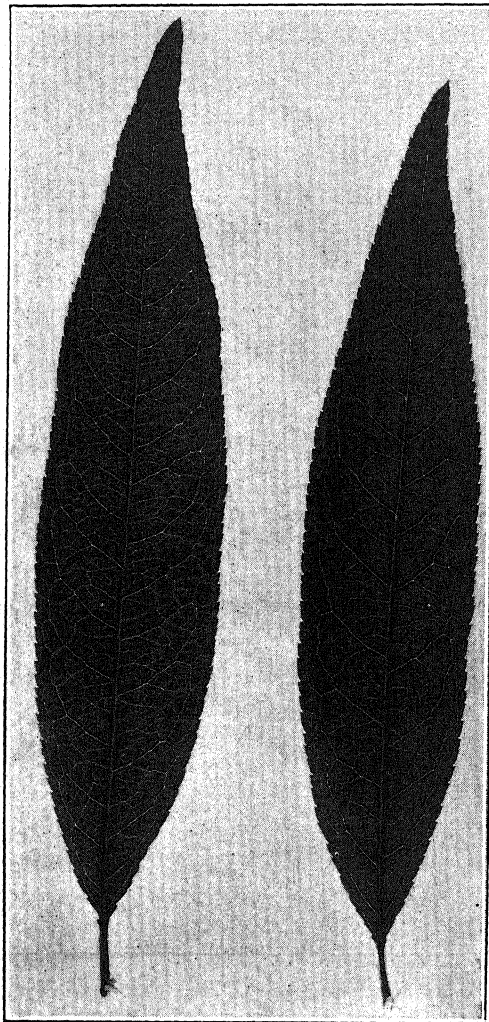


FIG. 2. Two peach leaves from the same tree. The leaf on the left shows clearing-of-veins caused by the yellows virus, while the leaf on the right is normal.

Yellows

An early symptom of the yellows disease is clearing-of-veins in leaves near the tips of young branches. The clearing is not so marked as to be conspicuous, but can usually be seen easily if observations are made at the proper time on rapidly-growing trees. Figure 2 shows 2 leaves of approximately the same size and age taken from the same tree 68 days after it was inoculated with yellows virus. The clearing-of-veins symptom shown by the leaf on the left first became visible about 3 weeks before the picture was taken. The leaf on the right is healthy in appearance.



FIG. 3. Two peach seedlings of the same age. The tree that has produced the willowy type of growth characteristic of yellows in its late stages was inoculated 9 months before the photograph was taken. The tree on the right is healthy.

A second early symptom appears in very young leaves as they develop at the tips of infected branches. Such leaves, when from 6 to 10 mm. long, stand straight in branches that are healthy, but bend over to form sickle-shape structures in branches that are diseased. The production of numerous thin upright-growing shoots bearing small slightly chlorotic leaves is a conspicuous symptom of the yellows disease after it is well established. A healthy and a yellows tree grown from seeds planted on the same date are shown in figure 3. Although the disease is invariably lethal, young trees frequently live for 1 to 2 years after their infection.

Rosette

Trees infected by the rosette virus may show either of 2 distinct types of symptoms. They may wilt suddenly and die, as in the case of the tree shown in figure 4, or they may develop the well-known rosette type of growth pictured in figure 5. This growth is characterized by slight elongation of stems and branches and by progressive dwarfing of the leaves produced after infection. Clearing-of-veins and thickening of veins accompany the development of other rosette symptoms. Young leaves and twigs of rosette trees are dark green. The severity of the disease varies with the season of the year, the point of inoculation, and the age of the tree. If rapidly-growing, young trees become infected from inoculations made some distance above the soil level during the spring and early summer, they almost always wilt and die without producing rosettes. If, however, the trees are inoculated at the ground level, they usually produce the rosette type of growth, even though they are young and growing rapidly. If trees are large and growing slowly, they do not wilt without first producing rosettes, even when inoculations are made in their tops. Trees infected shortly before they become dormant invariably produce rosettes when growth is resumed after the rest period. All rosette trees wilt and die after a short period of time. They seldom live for more than a few months.

EXPERIMENTAL

The cross-immunity experiments reported below were designed to show whether trees infected with any one of the 3 peach viruses would acquire immunity from either of the other 2.

Inoculation of Yellows Trees and Little-Peach Trees with Rosette Virus

Experiments in which trees having yellows and trees having little peach were inoculated with rosette virus have brought no evidence that either the yellows or the little-peach disease gives any protection against rosette. When rosette buds are inserted in yellows trees or in little-peach trees, the

trees come down with rosette disease as promptly as do healthy ones. When subinoculations are made from any part of these trees to healthy seedlings, the rosette disease is transmitted. This proves that rosette virus quickly invades tissues infected by either yellows or little peach. The rosette disease affects trees so severely that it has not been possible to determine whether either the yellows disease or the little-peach disease is transmitted by these subinoculations, but it is clear that neither yellows nor little peach gives protection against rosette.

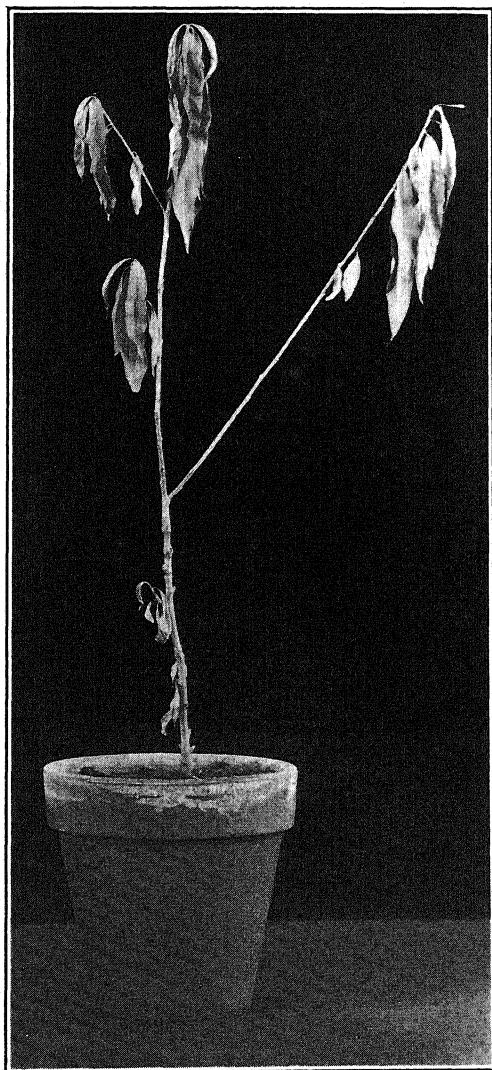


FIG. 4. A peach seedling that shows wilting without other rosette symptoms.

In one of these experiments, 5 trees belonging to a group of 10, that had had yellows for about 6 months and were thoroughly diseased, and 5 trees of another group of 10, that had had little peach for the same period of time and were diseased throughout, were inoculated with rosette virus by the insertion of rosette buds in their tops. At the same time, 5 out of a group of 10 healthy trees that were of the same age as the yellows trees and little-peach trees were similarly inoculated. The trees were held under observation during a period of 4 months following the rosette inoculations. All of the trees began to show the symptoms of rosette disease in about 5 weeks

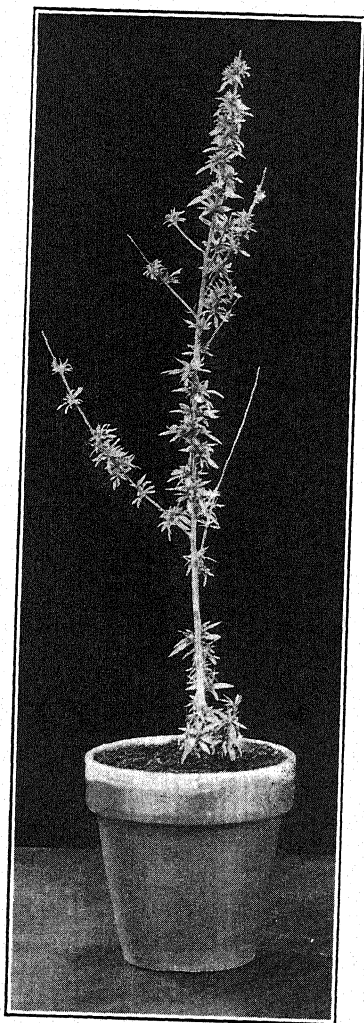


FIG. 5. A tree showing the usual rosette symptoms.

after they were budded. The rosette type of growth was produced at the tips of branches and the bark of all trees became a deeper green color than it had been previous to the rosette inoculations. Subinoculations to healthy trees from each of the 5 yellows trees and from each of the 5 little-peach trees 4 months after they were inoculated with rosette virus transmitted rosette disease. Symptoms developed by the trees gave no indication of transmission of yellows or little peach. This is not interpreted as evidence that the yellows and little-peach diseases were not transmitted together with the rosette disease. The symptoms of both yellows and little peach appear in the new growth that is produced after trees become infected. Rosette checks growth to such an extent that the symptoms of these diseases cannot develop. The experiments prove that rosette virus readily invades trees that have either the yellows or the little-peach disease, and that neither disease gives protection against rosette.

Reciprocal tests in which trees having rosette disease were inoculated with yellows virus or with little-peach virus have not yielded evidence that these viruses invade rosette tissues. This is not interpreted as proof that rosette gives protection against yellows and little peach, for the rosette trees never grow sufficiently to develop symptoms of either yellows or little peach. Yellows buds and little-peach buds transplanted to rosette trees take on the same deep green that is characteristic of the bark of rosette trees. All shoots produced by such buds show rosette symptoms. These tests bring further evidence that rosette virus readily invades tissues affected by either the yellows or the little-peach disease.

Inoculation of Trees Having Little Peach with Yellows Virus

Many experiments have been made in which trees having the little-peach disease were inoculated by inserting in their stems or branches buds from yellows trees. The yellows buds have lived and have, in many instances, produced shoots, but in no case have they transmitted the yellows disease. In a typical experiment, 10 healthy young trees about 3 feet tall and of the same age were inoculated with little-peach virus by the insertion of a bud in each tree at a point about 15 inches above the soil level. Six similar trees were left uninoculated. The buds all lived and transmitted the little-peach disease to the trees. Two months after inoculation of these 10 trees, 8 of them were reinoculated by the insertion of a yellows bud in the stem of each at a point about 7½ inches above the soil level. Two of the healthy trees were similarly inoculated with yellows at the same time. The other 4 healthy trees were not inoculated. All of the buds lived. Yellows was transmitted to the 2 healthy trees, but was not transmitted to any of the 8 trees having the little-peach disease. In order to subject the immunity of the trees to a severe test, they were inoculated with yellows a second time

by the insertion of a yellows bud in the stem of each tree at a point about 12 inches above the soil level 2 months after the first yellows inoculations were made. A yellows bud was inserted in the stem of each of 2 of the healthy trees at this time. The other 2 healthy trees were left uninoculated. All of the buds lived. The 10 trees inoculated with little-peach virus showed the symptoms of little-peach disease in less than 2 months after they were budded, and they continued to show these symptoms until the experiment was ended, 15 months later. Although 8 of these trees were inoculated with yellows on 2 occasions, they exhibited only symptoms of little-peach disease. Their appearance was in every respect like that of the 2 trees that were inoculated with little-peach virus alone. The 4 trees inoculated only with yellows virus developed typical symptoms of yellows within 50 days after they were budded. The experiment proves that trees having little-peach disease are immune from yellows.

Shoots from Yellows Buds Used in the Inoculation of Trees Having Little-peach Disease

Mention already has been made of the fact that yellows buds transplanted to trees having the little-peach disease sometimes produce shoots. It is interesting to note that such shoots never show any of the symptoms of yellows, but invariably show typical symptoms of little-peach disease. When sub-inoculations are made from these shoots to healthy trees, the symptoms obtained are those of little peach and not those of the yellows disease. These statements are supported by results from a number of different experiments, one of which will be described.

A little-peach bud was inserted in the stem of each of 5 healthy young peach trees about 4 feet tall at points approximately 2 feet above the soil level. Two similar healthy trees were left uninoculated. The buds all lived and the trees into which they were transplanted all showed typical symptoms of little-peach disease in less than 2 months after their inoculation. Eight months after the 5 trees were inoculated with little-peach virus, they were reinoculated by the insertion of 5 yellows buds in the stem of each tree at points less than 2 feet above the soil level. At the same time 5 yellows buds were inserted in the stem of one of the healthy trees. The other healthy tree was left uninoculated. Two weeks after the insertion of yellows buds, the trees were cut back in order to force the transplanted buds into growth. All shoots except those from yellows buds were broken off as soon as they appeared. The uninoculated tree was cut back also, but the shoots that grew from its stem were not removed. All of the yellows buds lived and one or more in each tree produced shoots. A total of 11 shoots grew from the 25 yellows buds in the 5 trees having little-peach disease. The shoots were kept under observation over a period of 4 months. During this time they grew

to the following lengths: one to 9 inches, one to 18 inches, two to 20 inches, one to 21 inches, one to 27 inches, two to 28 inches, two to 32 inches, and one to 33 inches. Two of the 5 yellows buds inserted in the healthy tree also produced shoots; one was 14 inches and the other 17 inches long when the experiment was ended. Five shoots grew from the healthy tree. They reached lengths of 13, 18, 23, 25, and 37 inches, respectively. The shoots from the uninoculated healthy tree appeared healthy at all times, as was to be expected. The shoots produced by 2 of the yellows buds inserted in the other healthy tree showed the typical symptoms of yellows as soon as they pushed out. They bore small narrow leaves and numerous upright-growing side branches having short internodes. Yellows was transmitted to this tree. The 11 shoots that grew from the yellows buds inserted in the 5 trees having little-peach disease showed only symptoms of little peach. They bore broad, deep-green leaves of approximately normal size, and produced an undue number of short side branches, as is typical for the little-peach disease. The experiment shows that when yellows buds, which would transmit yellows to healthy trees and would produce shoots bearing typical symptoms of yellows if inserted in healthy trees, are transplanted to trees having little peach, they produce shoots bearing the symptoms of little peach and none of the symptoms of yellows. This suggests that, under the conditions of the experiment, the little-peach virus displaces the yellows virus in the tissues of transplanted buds.

That shoots from yellows buds transplanted to trees having the little-peach disease carry the little-peach and not the yellows virus was shown by subinoculations to healthy trees. Buds from the 11 shoots that grew from yellows buds inserted in the 5 little-peach trees mentioned in the experiment described above were transplanted to the stems of 25 healthy young trees. At the same time a little-peach bud was transplanted to each of 3 similar healthy trees. Three other healthy trees were left uninoculated. Two weeks after this operation, 15 of the 25 test trees were cut back to within 1 inch of the inserted buds. The 3 trees budded with little-peach buds were likewise cut back to within an inch of the inserted buds. The other 10 test trees and the 3 healthy trees were not cut back. As soon as new shoots started to grow from the stems of the cut trees, they were all removed except those from transplanted buds. The buds in the 10 trees that were not cut back did not produce shoots, but they transmitted the little-peach disease to every tree. The 15 shoots from the buds in the test trees that were cut back grew rapidly and bore typical symptoms of little-peach disease. After they had reached a length of 6 or more inches, 1 or 2 other shoots were allowed to grow from the stems of each of the 15 trees. These shoots likewise showed the symptoms of little peach. The shoots produced by these trees were identical in appearance with those derived from the 3 little-peach buds that were

inserted in healthy trees. The experiment proves that subinoculations from shoots produced by yellows buds after they are inserted in little-peach trees transmit the little-peach and not the yellows virus. It also proves that shoots produced by the buds used in making the subinoculations bear only the symptoms of little peach. The subinoculations bring further evidence that the little-peach virus displaces yellows virus in buds transplanted to little-peach trees.

Inoculation of Trees Having Yellows with Little-peach Virus

When buds from little-peach trees are inserted in healthy trees, they regularly transmit the little-peach disease, but when inserted in yellows trees they invariably fail to transmit this disease. Trees having the yellows disease are fully protected against little peach. In an experiment designed to test the immunity of yellows trees from little-peach disease, 16 healthy young seedlings about 3 feet high and of the same age were selected for uniformity in size and vigor. Ten of the trees were inoculated with yellows by inserting a bud from a yellows tree in the stem of each at a point about 18 inches above the soil level. The other 6 trees were left uninoculated. All of the budded trees came down with yellows within 2 months after their inoculation. Seven months later when the trees were thoroughly diseased, 5 of them were inoculated with the little-peach virus by inserting 4 little-peach buds in the stem of each tree at points varying from 20 to 25 inches above the soil level. Since some transplanted buds fail to develop shoots even when trees are cut back, 4 buds were inserted in each tree in order to secure a fair number of these shoots and also in order to subject the trees to a severe test of immunity from little peach. At the same time 3 of the healthy trees were inoculated with little-peach virus by inserting 4 buds in the stem of each tree at points from 20 to 25 inches above the soil level. The other 3 healthy trees were left uninoculated. Ten days after the insertion of the buds, all trees were cut back to within about 30 inches of the soil level. This was done in order to force out shoots and to promote the growth of inserted buds. All of the transplanted buds lived. The trees were kept under observation during a period of 6 months following the insertion of little-peach buds. The 3 trees inoculated only with little-peach virus came down with little-peach disease in about 6 weeks after the buds were inserted. The 5 yellows trees that were similarly inoculated showed no symptoms of little peach up to the time the experiment was ended. The appearance of these 5 trees was at all times exactly like that of the 5 yellows trees that were not inoculated with little-peach virus. The results are identical with those obtained in several other experiments in which length of time interval between date of inoculation with yellows virus and date of inoculation with little-peach virus was varied. The experiments prove that yellows trees are immune from the little-peach disease.

Shoots from Little-peach Buds used in the Inoculation of Trees Having Yellows Disease

Seventeen of the 20 little-peach buds that were inserted in the 5 yellows trees mentioned in the experiment described above produced shoots, and 8 of the 12 little-peach buds inserted in the 3 healthy trees mentioned in the same connection likewise produced shoots. The shoots from the little-peach buds in the healthy trees all showed typical symptoms of little peach, but the 17 shoots produced by the little-peach buds that were inserted in yellows trees all showed only symptoms of yellows. Their appearance was exactly like that of the other yellows shoots. However, in order to determine whether, in spite of their appearance, the shoots might carry little-peach virus, subinoculations were made from each of them by budding to a healthy tree. At the same time a bud from each of the 5 trees that had been inoculated only with yellows was inserted in each of the 5 other healthy trees. All of the buds lived, and they all transmitted the yellows disease. No differences were observed in the symptoms shown by the 2 sets of trees. The experiment proves that little-peach buds transplanted to yellows trees not only fail to transmit little peach, but apparently lose the virus of this disease. This suggests that, under the conditions of the experiment, yellows virus is capable of displacing little-peach virus in transplanted buds. Shoots taken from some of the trees used in experiments described above are shown in figure 6. The shoot marked A is from a yellows bud that was transplanted to a healthy tree. The shoot marked C is from a similar yellows bud that was transplanted to a little-peach tree. Shoot A shows the symptoms of yellows, while C bears the symptoms of little peach. Shoot B grew from a little-peach bud that was transplanted to a healthy tree, while shoot D grew from a similar bud transplanted to a yellows tree. Shoot B bears typical symptoms of little peach, while shoot D shows the symptoms of yellows. The symptoms of the 2 diseases are not satisfactorily portrayed by the pictures because they do not bring out color differences, but it is easy to see that shoot A looks like shoot D and that shoot B looks like shoot C. The waviness shown by some of the leaves on shoot C is due to the fact that this shoot wilted slightly before the picture was made.

Further Evidence of Displacement of One Virus by Another

The fact that yellows buds transplanted to little-peach trees produce shoots that bear the symptoms of little peach, and that little-peach buds transplanted to yellows trees produce shoots that bear the symptoms of yellows, indicates that, under suitable conditions, either virus is capable of displacing the other in infected tissues. Since, however, new shoots are produced from cells in the growing points of the buds and there is at present no way of determining whether or not these cells are infected at the time the

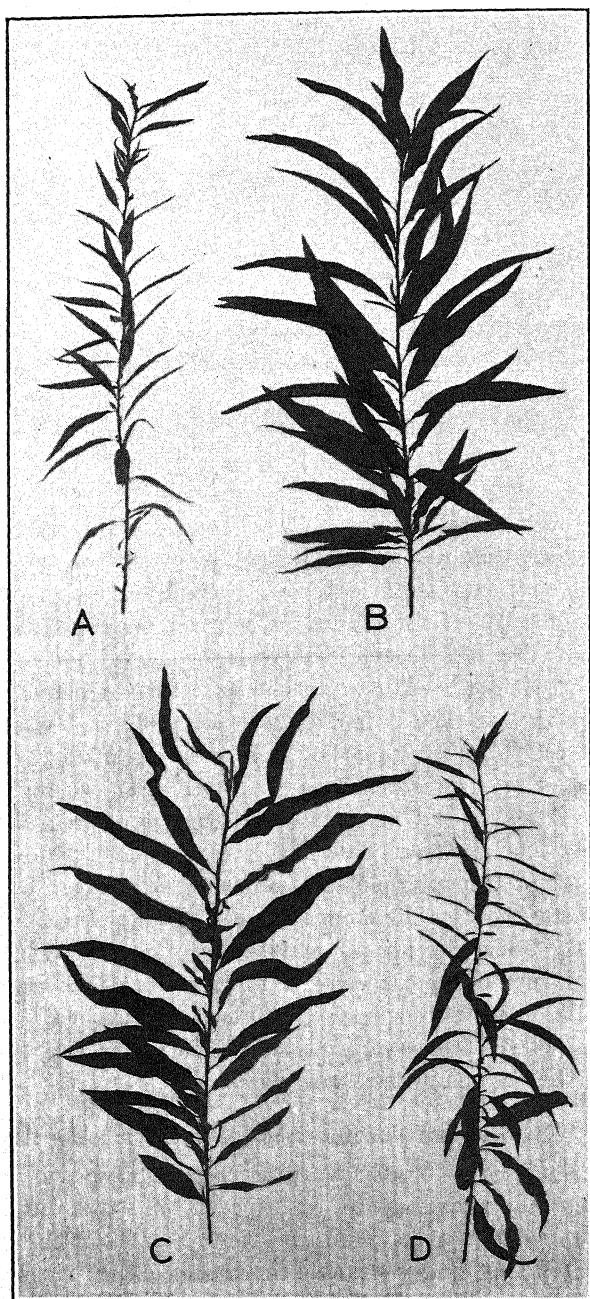


FIG. 6. Four shoots that grew from transplanted buds. Shoots A and C are from yellows buds that grew in healthy and in little-peach trees, respectively, while shoots B and D are from little-peach buds that grew in healthy and in yellows trees, respectively. Shoots C and D show the symptoms of the diseases carried by the trees in which they grew

buds are transplanted, it seemed possible that virus from the trees in which the buds were inserted might move into the new growth without displacing the other virus in cells of the older tissues of the buds. In order to obtain evidence on this point, 3 yellows buds that had been transplanted to little-peach trees and had grown in these trees for a period of 11 months without producing shoots, and 3 little-peach buds that had grown in yellows trees for the same period and had likewise produced no shoots, were carefully removed. Their edges were trimmed sufficiently to insure that no bark from the trees on which they had grown would adhere to the buds. One bud was then inserted in each of 6 healthy trees. The object of the test was to determine whether any given bud would transmit yellows virus, little-peach virus, or a mixture of the 2 viruses. The trees were under observation during a period of 4 months following their inoculation. The 3 inoculated with little-peach buds developed marked symptoms of yellows, and the 3 inoculated with yellows buds developed equally marked symptoms of little peach. There was no symptomatological evidence of a mixture of the 2 viruses in any of the 6 trees. The experiment proves that a yellows bud, that would transmit yellows to any healthy tree into which it might be inserted, will transmit the little-peach and not the yellows virus if it is transplanted and allowed to grow in a little-peach tree for a period of 11 months previous to its insertion in the healthy tree, and, likewise, that a little-peach bud capable of transmitting the little-peach virus to any healthy tree will transmit the yellows virus and not the little-peach virus if transplanted and left to grow in a yellows tree for the same period of time previous to its insertion in the healthy tree. These results support the view that, under suitable conditions, either of the viruses may displace the other, even in old tissues that are thoroughly invaded. It is, of course, possible that in removing the buds from the diseased trees into which they had been transplanted, a few cells from these trees may have adhered to the inner surfaces of the buds, but this seems unlikely since microscopic studies on stained sections through transplanted buds show that new cambium regularly develops from the bark side of the union and not from the stem tissues to which the transplanted buds adhere.

Dominance of One Disease over the Other Determined by Point of Inoculation

When it was found that yellows trees are immune from little peach, and that little-peach trees are immune from yellows, experiments were undertaken for the purpose of determining whether mixed infections could be obtained by the simultaneous inoculation of healthy trees with the 2 viruses. Seedlings were inoculated by inserting a little-peach bud and a yellows bud in their stems at convenient distances above the soil level. One bud was usually inserted about 12 inches and the other from 13 to 15 inches above

the surface of the soil. In some instances the yellows bud was placed at the 12-inch level and the little-peach bud at a higher level, while in other cases the position of the 2 buds was reversed. These experiments yielded unexpected results. The trees always came down with the disease carried by the bud placed in the uppermost position. When the yellows bud was inserted at the 15-inch level and the little-peach bud at the 12-inch level, the trees always came down with the yellows disease, but when the position of the buds was reversed they always came down with the little-peach disease. One or both of the transplanted buds frequently produced shoots. Any shoot that grew from a bud in the uppermost position always showed the symptoms of the disease carried by that bud, but any shoot that grew from a bud in the lower position showed the symptoms of the disease carried by the upper bud and no symptoms of the disease carried by the bud from which the shoot grew. In one of these experiments, 12 healthy young trees were inoculated with both the yellows and little-peach viruses. The yellows buds were inserted above the little-peach buds in 6 of the trees and below the little-peach buds in the other 6 trees. The lower buds were placed about 12 inches and the upper buds about 14 inches above the soil level. Six similar trees served as controls. Two of these were inoculated with little-peach virus only, 2 with yellows virus only, and 2 were left uninoculated. The trees were kept under observation during a period of 15 months following inoculation. All of the buds lived. The 6 trees in which yellows buds were inserted above little-peach buds came down with yellows within $2\frac{1}{2}$ months after their inoculation. The 6 trees in which yellows buds were inserted below little-peach buds all came down with little peach. The 2 control trees inoculated only with yellows virus and the 2 inoculated only with little-peach virus came down with yellows and with little peach, respectively, within about the same period of time. Seven of the 12 buds placed at the lower level in the 12 test trees produced shoots varying from 12 to 20 inches in length. Four of these carried the little-peach virus and 3 carried the yellows virus when they were inserted. The 4 that carried the little-peach virus produced shoots bearing symptoms of yellows, and the 3 that carried the yellows virus produced shoots bearing the symptoms of little peach. Subinoculations from the shoots with yellows symptoms resulted in the transmission of the yellows disease, while subinoculations from shoots with little-peach symptoms gave the little-peach disease.

The experiment proves that point of inoculation determines which virus will prevail in any given tree to which both are transferred on the same date. Here again the results suggest that either virus is capable of displacing the other. Results similar to those reported above were obtained even when the 2 buds were not inserted on the same date. When a bud was transplanted to the upper position either a few days before or a few days after that placed in the lower position, the trees always came down with the disease carried by the bud

in the upper position. When, however, the interval between the dates on which the 2 buds were inserted was as much as 3 weeks, the trees frequently showed symptoms of both diseases, provided the buds placed in the lower position were inserted first. In such cases the disease carried by the upper buds affected the upper parts of the trees, while that carried by the lower buds affected the lower parts of the trees. It seems that the virus carried by the upper bud does not prevail if that carried by the lower bud is sufficiently well established in the base of the tree when the other bud is inserted in the upper position.

DISCUSSION

The inoculation of yellows trees and little-peach trees with rosette virus always causes infection and the virus spreads with about the same rapidity in such trees as in healthy ones. Moreover, there is no evidence that the rosette disease loses any of its severity in either yellows trees or little-peach trees. In these respects the behavior of the rosette virus when inoculated into trees affected by yellows or little peach appears to stand in sharp contrast to the behavior of yellows virus when inoculated into trees affected by little peach or the behavior of little-peach virus when inoculated into trees affected by yellows. The immunity tests bring no evidence of a close relationship between rosette and either of the other diseases.

Since yellows trees are immune from little peach and little-peach trees are immune from yellows, it is concluded that these 2 diseases are closely related, and that little peach should be classified as a mild strain of yellows. The fact that little peach appeared about 100 years later than yellows would suggest that it may have arisen as a variant of the latter.

It is interesting to note that the immunity reactions shown by the peach indicate a close relationship between two virus diseases that can not be mechanically transmitted except by tissue transplantation. Up to the present time the serological technique has not been successfully employed in demonstrating relationships between such diseases. Plant immunity tests are, therefore, especially valuable in taxonomic studies on diseases of this type.

The immunity reactions described above can not be used at present in controlling either little peach or yellows, because, although little peach is less severe than yellows, both ruin affected trees. Practical use of immunity reactions must await the finding of a strain of virus so mild as to cause no serious damage to either trees or fruits.

The most surprising result of the cross-immunity tests with little-peach and yellows viruses is the observation that either may apparently displace the other from infected buds. The fact that shoots bearing yellows symptoms are produced by little-peach buds inserted in yellows trees, and shoots bearing little-peach symptoms by yellows buds transplanted to little-peach trees, brings evidence that this occurs. The same phenomenon was ob-

served in trees inoculated with both viruses simultaneously by means of buds placed at different levels. When yellows virus is carried by the bud in the upper position the dominance of yellows symptoms might be explained by assuming that the severe disease, yellows, obscures the symptoms of the mild disease, little peach, but such an explanation becomes inadequate when the position of the buds is reversed and the mild disease caused by little-peach virus prevails over the severe disease caused by yellows virus. That a tree infected locally by a virus that would cause it to become severely diseased may be made to come down with a less severe disease by a subsequent inoculation with virus of the latter is a striking fact. Since shoots produced by the lower buds always bear symptoms of the disease carried by the upper buds, it is believed that the location of the points at which the two viruses are introduced determines in some manner which virus will displace the other. But the means by which these displacements are accomplished are not yet known. It is possible that the two viruses use the same materials for multiplication, and that the one present in the large member of the union (the tree) overgrows the one present in the small member (the transplanted bud). How the virus in a bud inserted at one level in a tree can replace the virus in another bud inserted at a lower level in the same tree is not explained by any theory involving mass action, for approximately equal doses of the viruses are used in making inoculations. Köhler (2) has reported that when tobacco plants infected with a weak strain of the Y-virus of potato are inoculated with a strong strain of the same virus, the latter displaces the former in the leaves at the top of the plant, and he has suggested that the weak virus is assimilated by the strong one ("... dass das schwache Virus von den starken vollständig unterdrückt, vielleicht sogar assimiliert wird.").

Both the little-peach and the yellows (3) virus move much more rapidly downward than upward in newly infected trees. It might be expected that virus in a bud inserted low down on the stem of a tree rather than virus in a bud inserted at a higher level would invade the lower portions of the tree. There is at present no explanation as to why the reverse occurs. It is conceivable that virus from the lower bud does invade the tree, and that it is not completely displaced by virus from the upper bud; but if this occurs the virus from the lower bud is present without producing symptoms that can be detected in young trees. In any case, the virus in the upper bud dominates.

It should be clearly understood that the experiments described in this paper do not prove that little-peach virus fails to invade yellows trees when little-peach buds are inserted in the trees, or that yellows virus fails to invade little-peach trees when yellows buds are inserted in these trees. All that can be said is that, if such invasions have occurred, they have not caused detectable modifications in the symptoms shown by the trees. Each disease protects against the other.

SUMMARY AND CONCLUSIONS

1. A description is given of the symptoms produced in young peach seedlings by the rosette, yellows, and little-peach viruses.

2. Rosette virus readily invades trees affected by either little peach or yellows and neither of these diseases gives any protection against rosette. Therefore, it is concluded that rosette is not closely related to little peach or yellows.

3. Trees having little-peach disease are immune from yellows, and trees having yellows are immune from little peach. Since invasion of trees by either virus protects them against the disease caused by the other, it is concluded that little peach and yellows are closely related and should be classified as strains of the same disease.

4. Yellows buds transplanted to little-peach trees produce shoots that show typical symptoms of little-peach disease, and little-peach buds transplanted to yellows trees produce shoots that show typical symptoms of yellows. Subinoculations from these shoots transmit the disease carried by the tree into which the bud was transplanted and not the disease carried by the bud at the time it was transplanted. Therefore, it is concluded that either virus may displace the other in infected tissues.

5. Trees inoculated simultaneously with both the little-peach and the yellows virus by means of buds inserted at different levels in their stems come down with the disease carried by the bud in the upper position. Any shoot produced by the bud in the lower position will bear the symptoms of the disease carried by the bud in the upper position. Subinoculations from such a shoot transmit the disease carried by the bud in the upper position. It is concluded that point of inoculation determines which virus will prevail in any given tree.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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EFFECT OF CROWN RUST ON THE COMPOSITION OF OATS¹

H. C. MURPHY²

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INTRODUCTION

The effect of crown rust (*Puccinia coronata avenae* Eriks.) on the yield and water economy of oats has been reported recently by the writer (7).³ The grain harvested in these earlier studies afforded excellent material for use in determining the effect of crown rust on the composition of the grain of varieties initially infected at different stages of development. Additional studies were conducted to determine the effect of crown rust on the yield and composition of green plants of susceptible and resistant varieties. Long (4) observed in 1919 that oat leaves, heavily infected with crown rust, contained 6.82 per cent of reducing substances, while rust-free leaves contained 15.24 per cent. Caldwell *et al.* (3) have made a rather complete study of the effect of leaf rust (*P. triticina* Eriks.) on the composition of wheat plants and grain. Otherwise, there is little published information regarding the effect of the various cereal rusts on the composition of their hosts. A preliminary report of the data presented herein has been published by the writer (6).

METHOD AND MATERIALS

In the spring of 1930, pure-line selections of Victoria (C.I.⁴ 2401) and Markton (C.I. 2053) were grown in the greenhouse in soil of uniform moisture and artificially inoculated with physiologic form 1 of crown rust at different stages of development. There were 50 1-gal. jars, each containing 4 plants of the Victoria selection and another set of equal numbers containing Markton plants. The plants in 10 jars of each set were dusted with an abundance of urediospores of crown rust when they were in the seedling (4-leaf) stage. A like number of each set was dusted in the early boot, early

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³ Reference is made by number to Literature Cited, p. 234.

⁴ C. I. refers to accession number of the Division of Cereal Crops and Diseases.

anthesis, and early dough stages, respectively, and each of the 4 lots was reinoculated every two weeks. The water requirement and the yield of grain, straw, and roots of these plants, as well as a more detailed description of the technique involved, have been published (7).

The grain harvested from the plants studied in 1930 was retained. In March, 1934, a sufficient quantity of this grain was carefully hulled by hand to allow 35 grams of kernels of each lot. The samples of kernels and hulls were sent to H. J. Brownlee of the Science Department, Quaker Oats Company, Cedar Rapids, Iowa, who kindly determined the 6 crude constituents: moisture, ash, protein, fat, fiber, and nitrogen-free extract.

On October 6, 1933, pure-line selections of the varieties Markton (C.I. 2053), Iogold (C.I. 2329), Victoria (C.I. 2401), and Bond (C.I. 2733) were planted uniformly in 1-gal. jars, with 6 jars for each selection. After emergence, the number of plants was reduced to 15 per jar. On December 22, 1933, the plants in half of the jars of each selection were inoculated uniformly in a manner similar to that used in 1930. The susceptible selections showed 100 per cent infection within 10 days. The same plants were reinoculated on January 5, 1934.

On January 19, 4 weeks after the first inoculation, samples of the infected and rust-free plants of each selection were taken for chemical analyses. At that time the rust-free plants were all in the boot stage and the Iogold plants were just at the point of showing first heading. All of the plants in the 3 jars of each lot were cut close to the soil line and the total green weight from each jar was recorded. Representative duplicate 100-gram samples were immediately cut into small pieces and dropped into boiling alcohol. Additional duplicate samples were obtained for moisture determination. The 100-gram samples were extracted with 80 per cent alcohol and the extract was made to standard volume. From this fraction, aliquot portions were taken for determination of alcohol-soluble solids, ash in extract, alcohol-soluble nitrogen, and sugars. After extraction the residue was oven-dried, weighed, and recorded as the alcohol-insoluble fraction. Portions of the residue were used for the determination of ash, alcohol-insoluble or residual nitrogen, and the polysaccharides. Moisture was determined by drying the green samples, taken for that purpose, to a constant weight in an electric oven at a temperature of 100° C. Soluble solids were determined by drying aliquot portions of the alcoholic extract in a vacuum oven at a temperature of 75° C., and total solids were obtained by adding the values of the soluble and insoluble fractions. Ash was determined by igniting the soluble and insoluble solids in an electric oven at a dull red heat until constant weight was obtained. The nitrogen of the residue was determined by the Kjeldahl method, and that of the extract by the reduced iron-powder method described by Pucher *et al.* (8). The value of the total

nitrogen was obtained by adding these two values. Ammonia, amide, and nitrate and nitrite nitrogens were determined by the methods described by Appleman *et al.* (1). Sucrose and glucose were determined by the volumetric permanganate Munson-Walker method, levulose by the Jackson method. An attempt was made to digest starch from the residue after the dextrin had been previously extracted with 10 per cent alcohol and hydrolyzed with hydrochloric acid. The acid-hydrolyzable polysaccharides in the residue from the starch determination were determined by boiling with hydrochloric acid.

All analytical data reported in this paper have been calculated on the air-dry-weight basis for the grain, and on the green- and dry-weight bases for the green plants. Unless otherwise stated, the methods of analysis used were those described in Methods of the Association of Official Agricultural Chemists (2). The yields recorded for the green-plant material represent in each case the average of 3 jars. The percentages of the different chemical constituents each represent the average of duplicate samples.

EFFECT OF CROWN RUST ON COMPOSITION OF GRAIN

The susceptible Markton plants initially infected in the seedling and boot stages failed to produce grain and thus prevented any study of the effect of early infection on the composition of the grain of very susceptible plants. Markton plants initially inoculated in the anthesis stage produced 54.7 per cent as much grain as rust-free plants, while the yield of resistant Victoria plants initially inoculated in the seedling stage was 52.5 per cent of that of the rust-free ones. Crown-rust infection brought about a marked decrease in the yield of grain and an increase in the percentage of hulls, on both susceptible and resistant varieties, although the effect was much greater on the susceptible one. The results of this phase of the study have been presented in detail in a previous publication (7).

The outstanding finding of the present phase of this investigation is that, although crown rust had a very pronounced effect on the yield of grain and the relative percentage of kernels and hulls, it had little effect on the crude chemical composition of either kernels or hulls (table 1). Other than for the crude protein and nitrogen-free-extract contents of the kernels, there appeared to be no consistent relationship between rust infection and composition of either kernels or hulls. All of the other differences were small, inconsistent, and probably not significant. There was, however, a consistent, although slight, increase in the percentage of crude protein and decrease in the percentage of nitrogen-free extract of the kernels in relation to earliness of infection, *i.e.*, the earlier infection appeared, the greater was the increase in proportion of protein and the decrease in proportion of nitrogen-free extract. These relationships hold for both the susceptible and

resistant varieties, although the differences, particularly for the nitrogen-free extract, are very small and the apparent relationship might be a result of chance arrangement. Duration of infection had more effect on the crude-protein and nitrogen-free-extract content of the kernels than did type of infection. Markton, initially infected during anthesis, showed an increase in crude protein content of the kernels of 4.3 per cent and a decrease in nitrogen-free-extract content of 0.3 per cent, while Victoria plants initially infected at the same time showed an increase and a decrease of 5.1 and 1.9 per cent, respectively. Victoria plants initially inoculated in the seedling stage showed an increase in crude protein content of the kernels of 12.1 per cent and a decrease in nitrogen-free extract of 3.6 per cent.

EFFECT OF CROWN RUST ON COMPOSITION OF GREEN PLANTS

The writer (7) has shown that crown rust materially reduces the yield of oat straw, although the reduction is not so great as that for the grain or roots. Oats often are harvested before maturity and used as a soiling crop or as a hay crop. In addition to the study of the effect of crown-rust infection on the yield and composition of the grain a study was made to determine the effect on yield and composition of the green plant. Because of the great reduction in yield of the susceptible varieties resulting from the rust infection, all the plants were harvested while these heavily infected plants were still green and in the boot stage of development. If harvest had been delayed until the milk stage, as was originally planned, the infected susceptible plants probably would have been dead. Under field conditions infections occur often as early in the plant's stage of development as that initiated in this experiment and the effect produced by the rust at this stage probably is as important as that at a later stage.

Markton, Iogold, Victoria, and Bond were used because they range in reaction from the completely susceptible Markton to the nearly immune Bond (5). Urediospores of physiologic form 1 were used for all inoculations. Markton and Iogold each showed an equal percentage of infection amounting to 100 per cent, while the amount of infection on Victoria and Bond was approximately 85 and 40 per cent, respectively. Markton was completely susceptible, showing large uredia with very slight chlorotic areas adjoining them. Iogold was slightly less susceptible than Markton, showing somewhat smaller uredia with more adjoining chlorosis. Victoria was very resistant, showing few minute uredia with heavy necrotic areas surrounding them, and much necrotic area without uredia. Bond was nearly immune, showing necrotic and chlorotic flecks only.

From a physiological standpoint the effect of the rust on the green plant is best shown by calculating the percentage of the various constituents on a green-weight basis, as they actually occurred in the living plant; while from

TABLE 1.—Effect of crown rust on the chemical composition of grain of oat varieties initially infected at different stages of development

Variety	Stage at initial infection	Yields	Portion of total grain	Moisture	Ash	Crude protein (N × 6.25)	Fat	Fiber	Nitrogen-free extract
		Grams	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
		Kernels							
Markton (Susceptible)	Anthesis	14.1 ± .5	68.6	6.1	2.3	16.9	6.7	2.3	65.7
	Control	25.8 ± .1	79.4	6.0	2.4	16.2	6.9	2.6	65.9
Victoria (Resistant)	Seedling	12.6 ± .2	70.8	5.9	2.4	22.2	6.1	2.6	60.8
	Boot	16.6 ± .4	72.1	5.5	2.5	21.5	6.3	2.4	61.8
	Anthesis	19.6 ± .4	73.9	6.1	2.4	20.8	6.3	2.5	61.9
	Control	24.0 ± .2	75.0	6.3	2.2	19.8	6.4	2.2	63.1
Hulls									
Markton (Susceptible)	Anthesis	31.4	3.6	7.1	1.8	0.8	34.4	52.3
	Control	20.6	3.9	7.3	2.6	0.7	31.7	53.8
Victoria (Resistant)	Seedling	29.2	3.1	7.4	4.2	0.8	31.4	53.1
	Boot	27.9	4.5	7.5	3.2	0.9	33.8	50.1
	Anthesis	26.1	4.1	7.1	3.0	0.4	32.8	52.6
	Control	25.0	4.4	7.2	3.4	0.8	31.9	52.3

^a Average yield of grain (kernels plus hulls) per jar, of 10 jars with four plants each.

a practical or feeding standpoint it is preferable to present the relative proportions of certain of the general constituents calculated on a dry-weight basis.

Yield

The average yields (green weight) of the infected Markton, Iogold, Victoria, and Bond plants were decreased 69.3, 63.6, 22.2, and 14.7 per cent, respectively, as a result of crown-rust infection (Table 2 and Figs. 1 and 2). The yield of these plants, expressed as dry weight, is shown in table 3. The amount of change calculated on a dry-weight basis is very similar to that obtained on a green-weight basis because there is little variation in the moisture content between the different samples. The relative susceptibility of each variety was reflected by the decrease in yield of the infected plants. It is obvious, however, that the differences between the effect on the resistant and susceptible varieties would be greater under natural field conditions because of the partial or complete inability of the pathogen to reproduce on the more resistant plants, consequently limiting the supply of inoculum available for secondary and later infections.

Moisture

The infected susceptible plants contained slightly less moisture than the rust-free ones, while the moisture content of the infected resistant plants was slightly higher than that of the rust-free plants. The differences are very slight and, although consistent, they are of doubtful significance. It is worthy of note, however, that if the percentage of moisture is ascertained in an entirely different manner, *i.e.*, by subtracting the total solids from the green weight, the same relationship exists and the comparative percentages thus obtained are apparently well within the range of experimental error.

Solids

Crown rust brought about a definite decrease in the percentage of alcohol-soluble solids in the plant. This decrease ranged from 19.3 per cent for Markton to 10.8 per cent for Bond. Whereas the soluble solids were decreased as a result of crown-rust infection, the proportion of alcohol-insoluble solids was increased in amounts ranging from 29.6 to 4.0 per cent for Markton and Bond, respectively. The total solids were slightly increased in the 2 susceptible varieties as a result of infection but decreased in the resistant Victoria and Bond varieties. The decrease in soluble and the increase in insoluble solids is, in part at least, a direct result of the effect of the rust on the percentage of nitrogen, sugars, and polysaccharides in the plants, as shown later.

Ash

Plants infected with crown rust showed regularly a greater total ash content than rust-free plants. The percentage of ash in both the extract

TABLE 2.—*Effect of crown rust on yield and composition of green plants of susceptible and resistant oat varieties (green-weight basis)*

Variety	Condi- tion of plants	Yield	Mois- ture	Solids		Ash		Nitrogen				Sugars			Polysac- charides		
				Total insol- soluble	Total insol- uble	Total soluble	Total insol- uble	Amide	Nitrate and ni- trite	Total soluble	Total insol- uble	Su- crose	Glu- cose	Levu- lose	Dex- trin	Acid hydro- lyzable	
				Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Markton (Susceptible)	Infected	81.07	81.33	5.62	13.52	1.54	1.26	.050	.047	.211	.319	.297	.40	.40	.03	.56	2.75
	Rust-free	264.08	82.29	6.96	10.43	.81	.67	.014	.011	.050	.084	.236	2.44	1.88	1.16	.73	2.12
Logold (Susceptible)	Infected	89.96	83.15	5.25	11.42	1.04	1.07	.039	.039	.230	.316	.254	.42	.35	.06	.64	2.36
	Rust-free	247.14	83.67	6.32	10.08	.76	.82	.011	.013	.055	.084	.224	2.55	1.74	1.13	.81	1.97
Victoria (Resistant)	Infected	172.05	83.31	5.37	10.74	1.56	.90	.013	.028	.050	.185	.288	.95	.45	.39	.50	1.99
	Rust-free	221.10	82.77	6.53	10.13	1.30	.74	.011	.010	.033	.088	.270	2.37	1.34	1.14	.60	1.90
Bond (Nearly immune)	Infected	235.97	85.03	5.70	9.20	1.03	.57	.011	.014	.026	.105	.248	2.54	1.41	.79	.61	1.87
	Rust-free	276.50	84.70	6.39	8.85	.94	.53	.011	.010	.025	.067	.239	3.15	2.02	1.17	.70	1.81

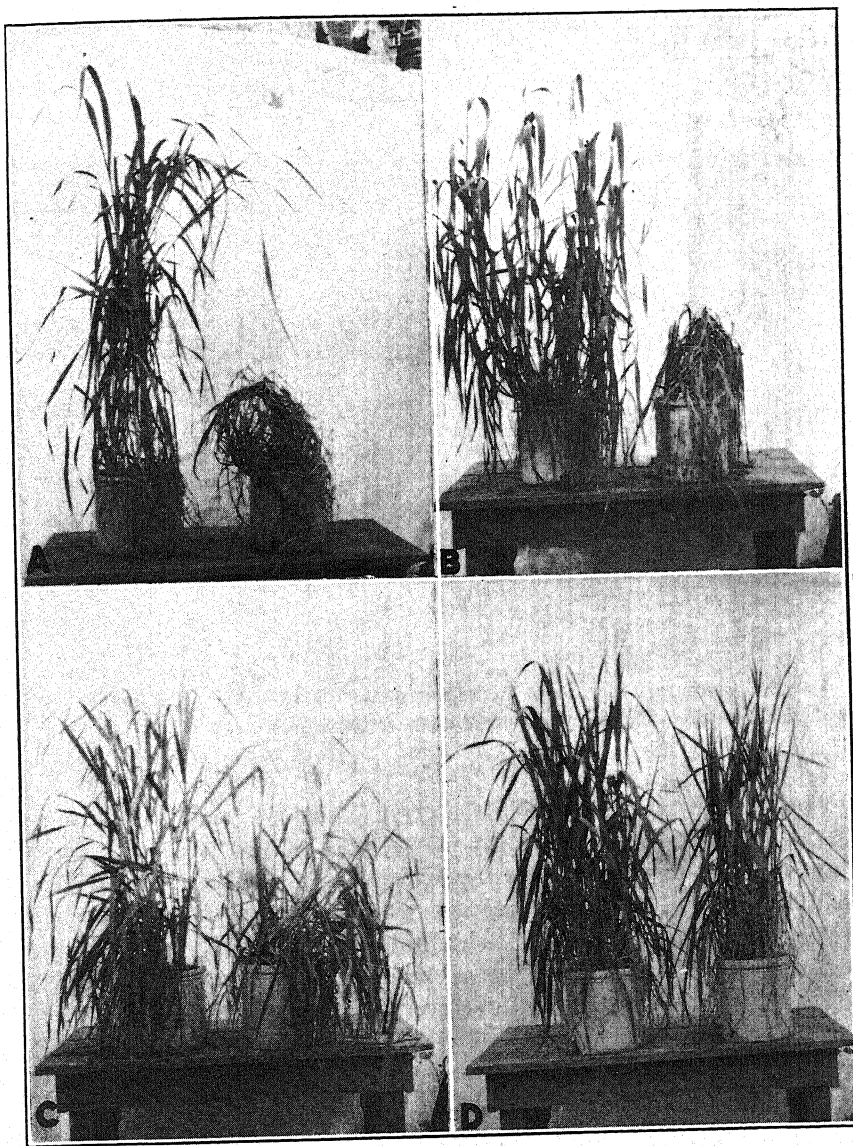


FIG. 1. A. Rust-free (left) and infected (right) plants of Markton. B. Iogold. C. Victoria. D. Bond.

and residue was increased as a result of the rust infection. Markton showed the greatest increases both in alcohol-soluble and alcohol-insoluble ash content, amounting to 90.1 and 88.0 per cent, respectively, while for Bond the increases were 9.6 and 7.5 per cent, respectively.

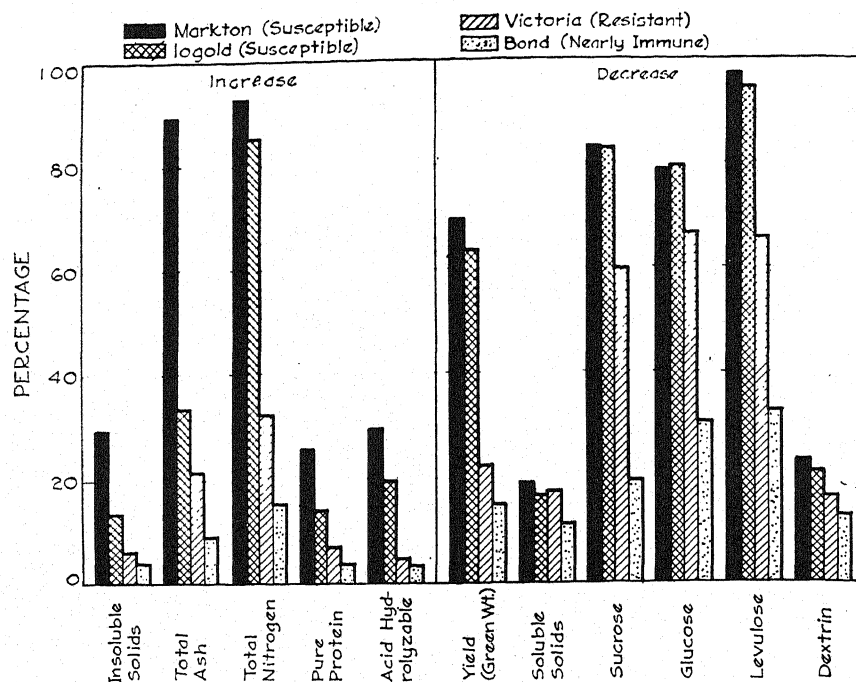


FIG. 2. Percentage increase or decrease in proportion of certain chemical constituents and of yield for infected green plants of Markton, Iogold, Victoria, and Bond oat varieties.

Nitrogen

The total nitrogen content in the green plants was small, amounting to less than one per cent. Infected plants, both of the susceptible and resistant varieties, contained a higher proportion of alcohol-soluble and alcohol-insoluble nitrogen than did rust-free plants. The difference was much greater for the soluble or noncolloidal nitrogen, amounting to 279.8, 276.2, 110.2 and 56.7 per cent, respectively, for the infected Markton, Iogold, Victoria, and Bond plants, while the corresponding increase in proportion of insoluble or colloidal nitrogen was 25.8, 13.4, 6.7, and 3.8 per cent. Striking increases in the percentages of ammonia, amide, and nitrate and nitrite nitrogens accounted for most of the increase in the noncolloidal nitrogen. The percentage of ammonia nitrogen was 257.1, 254.5 and 18.2 per cent greater in the infected plants of Markton, Iogold, and Victoria, respectively, than in the rust-free plants, while for the nearly immune Bond there was no difference. Amide nitrogen was increased 327.3, 200.0, 180.0, and 40.0 per cent, respectively, in the infected plants of these same varieties, while the corresponding increase in nitrate and nitrite nitrogen was 322.0, 318.2, 51.5, and 4.0 per cent. The percentage of nitrate and nitrite nitrogen was greater than that of the ammonia and amide nitrogen combined.

TABLE 3.—*Effect of crown rust on yield and composition of green plants of susceptible and resistant oat varieties (dry-weight basis)*

Variety	Condition of plants	Yield	Total ash		Total nitrogen		Total crude protein ^a		Alcohol insoluble protein ^b		Total sugars		Total polysaccharides		Total carbohydrates	
		Grams	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Markton (Susceptible)	Infected	15.14	15.00	3.30	20.63	9.94	4.45	17.73	22.18							
	Rust-free	46.77	8.36	1.81	11.31	8.33	30.94	16.09	47.03							
Iogold (Susceptible)	Infected	15.16	12.52	3.38	21.13	9.42	4.93	17.80	22.73							
	Rust-free	40.36	9.68	1.89	11.81	8.57	33.19	17.02	50.21							
Victoria (Resistant)	Infected	28.72	14.74	2.83	17.69	10.87	10.72	14.92	25.64							
	Rust-free	38.10	11.84	2.08	13.00	9.80	23.21	14.51	42.74							
Bond (Nearly immune)	Infected	35.32	10.69	2.36	14.75	10.35	31.66	16.57	48.23							
	Rust-free	42.30	9.61	2.00	12.50	9.76	41.44	16.40	57.84							

^a Total nitrogen $\times 6.25$.

^b Insoluble nitrogen $\times 6.25$.

From the standpoint of feeding value the increase in the ammonia, amide, and nitrate and nitrite nitrogen are not necessarily beneficial; in fact, they are more likely detrimental. On the other hand, the smaller increase in the colloidal or protein nitrogen in itself doubtless increases the feeding value of the infected plants. This apparent increase is, however, offset many times by the decrease in sugars and dextrin, not to mention the reduced yield of the infected plants.

The effect of crown-rust infection on the percentage content of total crude and alcohol-insoluble protein is shown in table 3. Although the total crude protein (total nitrogen $\times 6.25$) is almost doubled in the diseased Markton and Iogold plants, this is obviously not an accurate estimation of the actual protein content of the plants because of the large proportion of inorganic and non-protein nitrogen present in the extract, especially from the diseased plants. If we consider alcohol-insoluble or colloidal nitrogen only and calculate what might be termed "pure protein" by multiplying this amount by the factor 6.25, then the greatest increase in protein content is 19.3 per cent, in the infected Markton plants.

Since the rust mycelium and spores that still adhered to the plant were analyzed along with the host tissue, it is probable that the additional colloidal nitrogen or "pure protein" found in the infected plants was present mainly in the fungus. At least, the ratio of increase in the insoluble nitrogen (Table 2) appears to be in close relation to the relative susceptibility of the different varieties and thus to vary directly with mycelial development. On the other hand, it would appear more likely that the great increase in inorganic nitrogen was caused by the fungus interfering with metabolism and translocation.

Sugars

Of the total sugars determined in the rust-free oat plants, approximately one-half was sucrose, slightly more than one-fourth, glucose, and slightly less than one-fourth was levulose (Table 2). The effect of crown-rust infection on the percentages of these sugars in the susceptible plants was striking and in direct contrast to the effect on the ash and nitrogen content. The decrease in percentage content of sucrose, glucose, and levulose in Markton, as a result of rust infection, was 83.6, 78.7, and 97.4 per cent, respectively, while the corresponding decrease in Iogold was 83.5, 79.9, and 94.7 per cent. These two varieties were very susceptible, and, in addition to the infected plants containing considerable amounts of mycelium and adhering spores, additional quantities of mature urediospores were lost. From these studies there is no certain means of knowing whether the lower percentage of sugars in the infected plants was caused by increased respiration of the host, consumption by the fungus, or interference with photo-

synthesis caused by shading, mechanical obstruction, etc. Probably all of these factors were involved to some extent.

Very few spores were produced by the fungus on the resistant Victoria variety. Microscopic examination of cross sections of infected leaves also revealed much less mycelial growth in Victoria leaves than was found in the two susceptible varieties, although necrotic areas covered most of the leaf surface. Reductions in percentages of sugars in infected Victoria plants, ranging from 59.9 per cent for sucrose to 66.4 per cent for glucose, show that infection characterized by heavy necrosis has a pronounced effect on sugar content.

Considering the difference in percentage of infection on the varieties (Markton and Iogold 100 per cent, Victoria 80 per cent) it appears that there is a close relationship between the percentage of infection and the reduction of the various sugars. This would seem to indicate that the main factor affecting the amounts of these sugars is interference with photosynthesis, such as shading and destruction of chlorophyl. When different infection types are involved, percentage of infection does not measure the relative amounts of mycelium or spores present. The infection on Bond was characterized by chlorotic flecks and amounted to approximately 40 per cent of the leaf area. Apparently the reduction in sugars was about proportional to the percentage of infection as determined by these chlorotic areas. There was, however, very little if any mycelial development in these infected leaves and no spores. Absence of chlorophyl apparently accounted for the reduced amount of the sugars. The apparent correlation between total sugars and percentage of infection on the 4 varieties is also quite evident (Table 3).

Starch

No appreciable quantity of starch was found in either the rust-free or infected plants of any of the 4 varieties studied. Starch is a relatively unimportant constituent of the green oat plant.

Dextrin

The proportion of dextrin in the plants studied did not exceed 0.81 per cent. Crown-rust infection decreased it in amounts ranging from 23.3 per cent for Markton to 12.9 per cent for Bond.

Acid hydrolyzable

In contrast to the effect on dextrin, crown-rust infection brought about an increase in percentage of acid-hydrolyzable substances. This is in agreement with the increase in insoluble solids and probably represents an increased balance of pectic and hemicellulose materials as a result of the consumption or retardation in the formation of sugars, dextrin, etc., by the

fungus. The ratio of the amounts of reduction in these substances for the different varieties was dependent apparently upon their relative susceptibility as measured by both percentage and type of infection.

DISCUSSION

The data reported herein and in a previous publication (7) present a fairly complete picture of the effect of crown-rust infection on the yield, water requirement, and composition of susceptible and resistant oat varieties under greenhouse conditions. The amount of change brought about in yield, water requirement, and composition appears to be in direct relation to the duration and severity of infection. Crown rust affected significantly the yield and water requirement of both susceptible and resistant varieties when it appeared any time before the dough stage of development of the host. The longer the duration of infection the greater was the depreciation in yield, increase in water requirement, and change in chemical composition of the grain. The more severe the infection, as measured by percentage and type of infection, the greater also was the depreciation in yield, increase in water requirement, and change in chemical composition of the growing plant.

Crown rust reduced the yield of grain and roots of both susceptible and resistant varieties much more than that of the straw, which included the remaining parts of the plant. The effect on chemical composition of different parts of the plant was somewhat different. Green plants infected for one month and harvested at the boot stage of development showed the greatest change in composition due to the rust. The percentages of the sugars (sucrose, glucose, and levulose) were greatly decreased, along with a considerable decrease in dextrin, while the ash, nitrogen fractions, and acid-hydrolyzable substances were increased. The same trend was noted in the composition of the mature kernels from plants infected at different stages of development, *i.e.*, the nitrogen-free extract, which includes the sugars, dextrin, starch, etc., was slightly decreased, while the nitrogenous matter or crude protein was increased, although the differences were slight in comparison with those of the green plant. Crown rust apparently had no significant effect on the composition of the mature oat hulls.

That crown rust is located in the plant at the point where photosynthesis takes place gives it an excellent opportunity to avail itself of the newly synthesized foods before they are translocated to other parts of the plant. Certain of these, such as the sugars, were doubtless consumed by the fungus in respiration and, consequently, the total quantity of sugar and other carbohydrates was reduced, even though the mycelium and a small portion of the spores were included in the analysis. The rust organism must interfere with photosynthesis and other metabolic processes in many ways, such as mechanical interference brought about by rupturing the epidermis, ob-

structing the flow of liquids and passage of gases, reducing photosynthesis because of necrosis and chlorosis and by shading, etc., thereby limiting the amount of carbohydrates synthesized in the plant. This interference with photosynthesis, particularly for resistant varieties, probably is more injurious to the plant than the loss of the carbohydrates consumed by the fungus. The fact that the percentage of ash, insoluble solids, soluble nitrogen, and acid-hydrolyzable substances, such as cellulose, show relative increases is doubtless a result of smaller utilization of these substances by the fungus rather than to any actual increase due to the presence of the rust organism. Oats grown under field conditions and harvested for green foliage or for hay, in addition to having a considerable reduction in yield, also may be reduced greatly in feeding value because of crown-rust infection; while oats harvested for grain may show the effect of the rust mainly by a great reduction in yield, with little change in the feeding value of the grain.

Caldwell *et al.* (3) found that the percentage of crude protein in the grain of susceptible varieties of wheat was reduced significantly by heavy leaf rust infection under field conditions. Sucrose of the grain also was decreased, while the starch content varied inversely with the protein content. The results obtained in the present investigation, conducted under greenhouse conditions, are in agreement with theirs, except for the effect on the protein content of the grain. The percentage of crude protein in the kernels of susceptible and resistant oat varieties was slightly increased by heavy crown-rust infection, while the acid-hydrolyzable substances, which included the sugars, starch, and other carbohydrates, were slightly decreased. The authors cited obtained a higher percentage of nitrogen and a lower percentage of sucrose and reducing sugars also in the culms and leaves of rusted plants, which is in agreement with the results reported herein.

The data obtained in this investigation and another reported previously (7) emphasize the value of crown-rust resistant varieties, such as Bond and Victoria. Bond was resistant to all except 2 of the physiologic forms of crown rust collected in the years 1931-33, whereas Victoria was resistant to all of 37 forms collected in the period 1927-33. In addition, Bond is resistant to certain forms of loose and covered smut and Victoria apparently is resistant to the forms of loose and covered smut prevalent in the central and southern oat areas of the United States.

SUMMARY

Under greenhouse conditions crown-rust infection, initiated in the anthesis stage on Markton (susceptible) and in the seedling, boot, and anthesis stages on Victoria (resistant), had no appreciable effect on the amounts of moisture, ash, fat, and fiber contained in the kernels. There was a slight increase in the crude protein as a result of infection, the amount of increase

apparently depending more upon the duration than upon the type of infection. Nitrogen-free extract was slightly decreased also in relation to duration rather than to type of infection. The composition of the hulls removed from these kernels was not appreciably affected by infection in any of the stages.

The green weight of infected plants of Markton and Iogold (both susceptible) and of Victoria and Bond (resistant and nearly immune, respectively) was 69.3, 63.6, 22.2, and 14.7 per cent lower, respectively, than that of rust-free plants.

Concentrations of insoluble solids, ash, nitrogen, and acid-hydrolyzable substances in the green plant, were all increased as a result of rust infection, the amount of increase being in relation to the susceptibility of each variety. Ammonia, amide, and nitrate and nitrite nitrogen showed the greatest percentage of increase. The increase in these non-colloidal nitrogen fractions for the susceptible Markton variety were 257.1, 327.3, and 322.0 per cent, respectively, while the greatest increase for the colloidal or protein nitrogen was 25.8 per cent. Corresponding maximum increases for insoluble solids and acid-hydrolyzable substances were 29.6 and 29.7 per cent, respectively.

The proportions of total soluble solids, sugars, and dextrin in the green plant were all decreased as a result of infection. The sugars showed the greatest decreases, sucrose, glucose, and levulose being decreased 83.6, 78.7, and 97.4 per cent, respectively, in the infected Markton plants, while total soluble solids and dextrin showed a corresponding decrease of 19.3 and 23.3 per cent, respectively.

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REACTION OF CERTAIN VARIETIES AND SPECIES OF THE GENUS HORDEUM TO LEAF RUST OF WHEAT, *PUCCINIA TRITICINA*¹

C. O. JOHNSTON²

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INTRODUCTION

Varieties of barley have been used by many uredinologists to eliminate mixtures of leaf rust of wheat, *Puccinia triticina* Eriks. from cultures of stem rust, *P. graminis tritici* (Pers.) Eriks. and Henn. Complaints have occasionally been heard that leaf rust mixtures seemed to persist in some cultures despite the use of that method of purification. Field collections of leaf rust on barley, accompanied by the statement that the collector believed the rust to be leaf rust of wheat, occasionally also have been received by the writer. Most of these have proved to be the leaf rust of barley, *P. anomala* Rostr. One collection, however, was received from G. L. Peltier of the Nebraska Agricultural Experiment Station in 1927 that proved to be *P. triticina*. It gave a normal infection on seedlings of wheat and when transferred to the differential wheat varieties produced reactions typical for physiologic form 9. When transferred from wheat to seedlings of a local strain of White Hull-less barley, the infection produced was characterized by many small uredia surrounded by yellow necrotic areas. Thus there seemed some evidence that leaf rust of wheat could be propagated on certain varieties of barley.

An examination of the literature reveals that as early as 1911 Freeman and Johnson (3) stated that leaf rust of wheat attacked rye and barley to some extent, although Carleton (1) in earlier experiments had obtained negative results with barley. More recently, Stakman (9) stated that Schaal, a student in his laboratory, discovered that certain varieties of barley were moderately susceptible to *Puccinia triticina*. Waterhouse (10) reported in 1929 that *Hordeum murinum* and *H. maritimum* were susceptible to 2 physiologic forms of *Puccinia triticina* in Australia, while *H. jubatum* was immune; no mention was made of the reaction of varieties of cultivated barley. Hassebrauk (6) observed that form 14 of *P. triticina* caused

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stule formation on *H. bulbosum*, *H. maritimum*, *H. spontaneum*, and several varieties of cultivated barley, while *H. jubatum*, *H. murinum*, and *secalinum* were highly resistant or immune. In the United States, Mains (1) reported negative results or only a trace of infection when *H. deficiens*, *distichon*, *H. gussoneanum*, *H. intermedium*, *H. jubatum*, *H. murinum*, *nodosum*, *H. pusillum*, and *H. vulgare* were inoculated with *P. trititica*. Matter and Levine (2), working with *P. graminis secalis*, discovered that a large number of barley varieties exhibited varying reactions when inoculated with several physiologic forms of that fungus.

In view of the diversity of published reports, it seems desirable to record the results of some experiments conducted at Manhattan, Kansas, on the reaction of cultivated varieties and wild species of barley to the leaf rust of wheat during the period 1928 to 1934, inclusive. These experiments were undertaken to gain some knowledge of the distribution of botanical types in the genus *Hordeum* capable of supporting a reproductive infection of *Puccinia trititica*.

MATERIALS AND METHODS

Seed of the cultivated varieties was obtained from H. V. Harlan of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. Separate lots of seed were used for the experiments of 1928 and those of 1933. Seed of the wild species was furnished by W. M. Bever, Moscow, Idaho, G. A. Wiebe, Davis, Calif., and B. Bayles, Division of Cereal Crops and Diseases. That supplied by the latter was grown at the Arlington Experiment Farm, Rosslyn, Virginia.

The experiments were conducted in the greenhouse during the late fall and winter months at temperatures fluctuating between 65° and 85° F. An effort was made to keep night temperatures as near 70° F. and day temperatures as near 75° F. as possible. Six to ten barley seedlings were grown in 4-inch flower pots and inoculated about 8–10 days after emergence. In the experiments of 1928 the primary leaves of all seedlings were hand-inoculated, but in all later experiments seedlings in the two-leaf to four-leaf stage were first atomized with tap water and then inoculated by dusting with spores of the desired physiologic form. Inoculated plants were placed in moist chambers for 24 hours after which they were placed in full light on the greenhouse bench until ready for rust readings 10–14 days later. Inoculations with each physiologic form were repeated two or more times to make sure the proper reactions were being observed and recorded.

In making the rust readings, the infection produced by each physiologic form was recorded in terms of infection types as described by Mains and Jackson (8) in their studies on physiologic specialization of *Puccinia trititica* on wheat. The readings were based on the type of infection developed on the primary leaf although infection also sometimes was present on sec-

ondary leaves. Primary leaves often bore more and larger uredia than secondary leaves, although the latter also occasionally exhibited pustules as high as the third or fourth leaf. In some cases uredia developed on the primary leaf while only flecking was shown by secondary leaves.

EXPERIMENTAL RESULTS

Reaction of Cultivated Barley Varieties to *Puccinia triticina*

In 1928 seedlings of 51 varieties of cultivated barley, representing 18 botanical groups were inoculated with *Puccinia triticina*, physiologic form 9, as described above. The experiment was repeated twice during that season and the data recorded in table 1 are averages of the three readings. Perhaps the most important fact shown by this table is that none of the barley varieties was so susceptible to that form of leaf rust of wheat as were the wheat varieties Malakof and Regal, i.e., none of the barleys was completely susceptible. On the other hand, several varieties of barley were more susceptible than the wheat variety Democrat, and several were as nearly susceptible as Hussar. It is perhaps inaccurate to speak of this type of reaction as susceptibility, for it actually is a moderate type of resistance in which there is a copious production of uredia that are small, usually are surrounded by yellow necrotic areas, and are accompanied by considerable flecking. Nevertheless, a number of barley varieties were hosts sufficiently favorable to permit reproduction by the fungus (Fig. 1).

The data in table 1 show that pustule formation is not limited to varieties in any one botanical group but occurs also on certain varieties of several groups. Ten of the 18 botanical groups were represented by only one variety and 2 others by only 2 varieties each. This is entirely too few to give anything like a true picture of the reaction of the group as a whole. Three groups, however, were represented by enough varieties to give a fair indication of their general reaction. These were *Hordeum vulgare pallidum*, *H. vulgare coeleste*, and *H. intermedium cornutum*. In the first of these Pannier C.I. 1330 was the only variety showing a moderate degree of pustule formation (type-2 infection). Of the 10 varieties in the *H. vulgare coeleste* group, there were 4 that exhibited an infection of type 2. The *H. intermedium cornutum* group exhibited the nearest approach to true susceptibility of any of those tested. Although all varieties of this group showed a moderate resistance (type-2 infection), there was a heavier production of uredia and these were somewhat larger and freer from chlorotic haloes than the pustules produced on most varieties of other groups. Three varieties of *H. deficiens* showed considerable pustule development in 1928 but failed to do so consistently in later experiments.

Fresh seed of a selected list of varieties was obtained and seedlings were tested with 6 physiologic forms of *Puccinia triticina* during the winter of

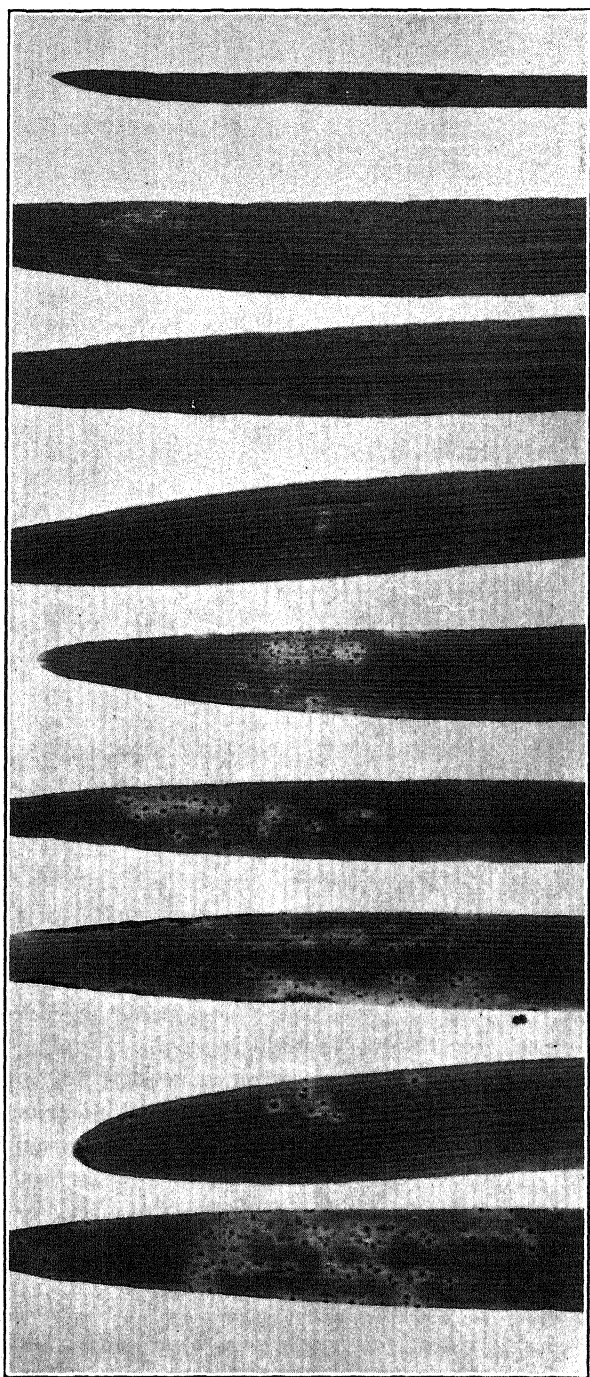


FIG. 1. Infections produced by *Puccinia trititica*, physiologic form 9, on the primary seedling leaves of eight varieties of barley and one variety of winter wheat in the greenhouse. The barley varieties from left to right are Cornutum C. I. 2366, Black Hull-less C. I. 666, Jet C. I. 967, Barley wheat C. I. 1384, Abyssinia C. I. 362, Hanna C. I. 203, Beldi Dwarf C. I. 190, and Oderbrucker C. I. 940. Winter wheat on the extreme right is Regal C. I. 7364.

TABLE 1.—*Reaction of barley and wheat varieties to physiologic form 9 of the leaf rust of wheat (Puccinia tritica) in the greenhouse. 1928*

Variety ^a	C. I. No. ^b	Rust infection type	Variety	C. I. No.	Rust infection type
<i>Hordeum vulgare pallidum</i>			<i>Hordeum vulgare</i>		
Beldi Dwarf	190	0	trifurcatum		
Club Mariout	261	0	Skinless	1032	0
Club Mariout	2334	0	Nepal	1376	0-1
Coast	626	0	Nepal	2322	0
Manchuria	245	0	<i>Hordeum intermedium</i>		
Mignon	999	0-1	nudihaxtoni		
Oderbrucker	940	0-1	Unnamed	2352	1
Odessa	927	0	<i>Hordeum intermedium</i>		
Pannier	1330	1-2	cornutum		
Trebi	936	0	Barley wheat	1384	2
<i>Hordeum vulgare nigrum</i>			Cornutum	2215	2
Gatami	575	0	"	2323	2
<i>Hordeum vulgare nigrum</i>			"	2427	2
leiorrhynchum			"	1317	2
Lion	923	0-1	"	2366	2
<i>Hordeum vulgare</i>			<i>Hordeum distichon palmella</i>		
horsfordianum			White Smyrna	658	0-1
Horsford	507	0	<i>Hordeum distichon palmella</i>		
<i>Hordeum vulgare coeleste</i>			nutans		
Ak Arpa	747	0-1	Chevalier	1419	0
Dehra	1085	0-1	Hanna	203	0-1
Hanseer Hull-less	703	2	<i>Hordeum distichon palmella</i>		
Hull-less	745	2	erectum		
India Hull-less	698	0-1	Svanhals	187	0
Kama-ore	694	2	<i>Hordeum distichon nigricans</i>		
Kharsila	2318	2	Black Smyrna	191	0-1
Semet	1314	0	<i>Hordeum distichon nudum</i>		
Yane-hadaka	693	0	Evans	621	0
Yane-hadaka	2319	0-1	<i>Hordeum distichon</i>		
<i>Hordeum vulgare coeleste</i>			nudumianthinum		
himalayense			Irisaka	1083	1-2
Ederle	1015	0	<i>Hordeum deficiens</i>		
Gorak	1086	1-2	nigrinudum		
Himalaya	620	0	Jet	967	1-2
<i>Hordeum vulgare coeleste</i>			<i>Hordeum deficiens steudelii</i>		
violaceum			Abyssinia	362	2-
Black Hull-less	1106	2	Steudelii	2226	1-2
"	596	0-1	<i>Triticum vulgare</i>		
"	666	1-2	Malakof	4898	4
"	1027	0-1	Hussar	4843	2
Spain	1370	0-1	Mediterranean	3332	0
			Regal	7364	4

^a Varieties classified according to Harlan (5).^b C. I. denotes accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

1933. The results obtained are recorded in table 2. These data show clearly that the three varieties of *Hordeum intermedium cornutum* that were tested approached susceptibility in several instances. This group as a whole and the variety Black Hull-less C.I. 666 of *H. vulgare coeleste*

TABLE 2.—*Reaction of a selected group of barley and wheat varieties to six physiologic forms of the leaf rust of wheat (Puccinia tritica) in the greenhouse. 1933*

Variety	C.I. No.	Type of infection ^a produced by form number					
		1	2	5	9	15	33
<i>Hordeum vulgare pallidum</i>							
Beldi Dwarf	190	0	0	0	0	0	0
Oderbrucker	940	0	0	0-1	0	0	0-1
<i>Hordeum vulgare coeleste violaceum</i>							
Black Hull-less	666	2-	2	2	2+	2-	2+
<i>Hordeum intermedium cornutum</i>							
Barley wheat	1384	2+	2+	2++	2+	2+	2+
Cornutum	2287	2	2	2+	2+	2+	2+
Cornutum	2366	2-	2-	2	2++	3-	2++
<i>Hordeum distichon palmella nutans</i>							
Hanna	203	0	0-1	0	0	0	0
<i>Hordeum deficiens nigrinudum</i>							
Jet	967	0	0	0	2-	0-1	0-1
<i>Hordeum deficiens steudeli</i>							
Abyssinia	362	0	0	0-1	0-1	0-1	0-1
Steudeli	2226	0-1	0	0-1	0-1	0	0-1
<i>Triticum vulgare</i>							
Malakof	4898	0	0-1	4	4	0	0
Hussar	4843	1	4	1	2-	2=	2=
Mediterranean	3332	0	4	4	0	4	4

^a Plus and minus signs are used to indicate tendencies toward a somewhat higher or lower infection type than the type indicated by the numeral. The sign of equality denotes a double minus.

violaceum were only moderately resistant to all six of the forms, while all other varieties were very highly resistant to all forms with the single exception that Jet C.I. 967 was only moderately resistant to form 9. Although there was some variation in the reaction of each of the three varieties of *H. intermedium cornutum* and the variety Black Hull-less to the various physiologic forms, the differences were not great enough to be of differential value or of much significance. The greatest difference in reaction was exhibited by Cornutum C.I. 2366 on which form 15 produced a 3-minus-type infection, while forms 1 and 2 produced only a 2-minus-type. The 3-minus infection was the nearest approach to true susceptibility observed in these studies. The variety Barley wheat C.I. 1384 exhibited the least resistance to all of the 6 forms, while Beldi Dwarf C.I. 190 was the most resistant to all. Despite the fact that Black Hull-less C.I. 666, Barley wheat C.I. 1384, Cornutum C.I. 2287, and Cornutum C.I. 2366 all bore infections in which

uredia were copiously produced when inoculated with the 6 physiologic forms, none of them could be considered susceptible to *P. triticina* in the same sense that the wheat varieties Malakof and Mediterranean were susceptible to some of the forms. They were, however, just as nearly susceptible as was the wheat variety Hussar to forms 9, 15, and 33, and much more nearly susceptible to form 1 than any of the 3 wheat varieties.

Reactions of Certain Wild Species of *Hordeum* to Physiologic
Forms of *Puccinia triticina*

Since some groups of the cultivated barleys seemed to be more favorable hosts for *Puccinia triticina* than others, it was decided to ascertain the response of certain species of wild barleys to several physiologic forms of that fungus. In the genus *Triticum* high susceptibility to leaf rust is most frequently encountered in the species having 42 somatic chromosomes, while those with lower chromosome numbers are more resistant. It seemed possible that the same thing might hold true for the genus *Hordeum*. Griffiee (4) has shown that all of the cultivated barley groups, *Hordeum spontaneum*,

TABLE 3.—Reaction of seedlings of several species of wild barley from various sources to the leaf rust of wheat (*Puccinia triticina*) in the greenhouse. Manhattan, Kans., 1934

Species	C.I. No.	Source of seed ^a	Infection type produced by form number		
			5	9	15
<i>H. murinum</i>	6069	California	0	0	0-1
" "		Idaho	2-	2	2=
" "	4102	Virginia	0	0	0
" "	4278	"	2=	2=	1
" <i>spontaneum</i>	2294	"	2=	2=	2=
" <i>gussoneanum</i>	6067	California	2	2	2-
" "	6066	"	1=	1	1
" <i>nodosum</i>	6072	"	0-1	0	0
" "		Idaho	0	0	0
" <i>maritimum</i>		"	2	1+	1+
" <i>bulbosum</i>	6071	California	0-1	2=	2-
" <i>jubatum</i>	6070	"	0	0	0
" "		Idaho	0	0	0
" <i>pusillum</i>	6078	California	0	0	0
" "		Kansas	0	0	0
" " <i>pubens</i> ...	6068	California	0	0	0

^a Seed was supplied by G. A. Wiebe, Davis, Calif., W. M. Bever, Moscow, Idaho, and B. B. Bayles, Div. of Cereal Crops and Diseases, United States Department of Agriculture, Washington, D. C. (seed from Arlington Experiment Farm, Rosslyn, Va.). The Kansas collection was made locally by the author.

H. maritimum and *H. Caput-Medusae* have only 14 somatic chromosomes, while *H. jubatum* and *H. murinum* have 28, and *H. nodosum* has 42.

Seed of species of wild barley was obtained from 3 different sources, and seedlings were grown in the greenhouse and inoculated with physiologic forms 5, 9, and 15 during the winter of 1934. The results obtained are given in table 3. The best development of uredia was obtained on *Hordeum gussoneanum* and two strains of *H. murinum*. On these the very numerous uredia were small and accompanied by chlorosis. It is interesting to note that both highly resistant and slightly susceptible strains appeared in *H. murinum* and *H. gussoneanum*. The California strain of *H. murinum* was definitely more resistant to all 3 rust forms than the Idaho strain, although the plants were morphologically indistinguishable. On the other hand the two strains of *H. gussoneanum* differed somewhat in certain plant characters as well as in their reactions to the three physiologic forms of leaf rust.

The reactions of the two strains of *Hordeum murinum* from Arlington Farm are of particular interest because differences between them had already been noted in plant characters. The strain C. I. 4278 was labeled "small seed" and the seedlings were definitely shorter in stature than those of the strain C. I. 4102. The small-seed type was a definitely more favorable host for all 3 physiologic forms than the large-seed strain. The latter exhibited nothing but flecking in all cases, while uredia of all forms developed on the small-seed type.

A few of the species seemed to have a slight differential reaction to the 3 physiologic forms of rust. For example, *Hordeum maritimum* was somewhat less resistant to form 5 than to forms 9 and 15, and *H. bulbosum* was more resistant to form 5 than to forms 9 and 15. In general, however, the differences were not distinct enough to be of much significance or of any differential value.

From the data obtained it seems clear that the nearest approach to susceptibility was not encountered in the species with the largest number of chromosomes (*Hordeum nodosum*). As a matter of fact that species was strongly resistant to all 3 physiologic forms. In the jubatum-chromosome group, which has 28 somatic chromosomes, *H. jubatum* was very highly resistant to all 3 forms, while *H. murinum* contained some strains that were very highly resistant and others that were only moderately resistant. Two wild species of the vulgare-chromosome group, having 14 chromosomes, were tested. One of these, *H. spontaneum*, was uniformly resistant to all forms but permitted a considerable development of uredia. The other, *H. maritimum*, was only moderately resistant to form 5 but rather highly resistant to forms 9 and 15. It is clear, therefore, that high resistance is found in all of the chromosome groups and that the weakest resistance appeared in the

groups having 14 and 28 chromosomes. The number of species tested is, however, too small to justify any general conclusions on the relation of chromosome number to resistance to *Puccinia triticina*. There is some evidence that a relationship exists and that the nearest approach to susceptibility can be expected in species with the lower chromosome numbers. Despite this fact, however, it must be kept in mind that very highly resistant and nearly susceptible varieties occur among the botanical groups of cultivated barleys all of which have 14 chromosomes.

DISCUSSION

The results of these studies would seem to indicate that no varieties of cultivated barley and none of the wild species of *Hordeum* are completely susceptible to the leaf rust of wheat. They also demonstrate that certain varieties and species approach susceptibility to an extent where there is a rather copious production of small uredia. Whether the propagation of leaf rust of wheat on barley would ever be of any economic significance is unknown to the writer. The data show that certain of the cultivated varieties and wild species of barley are capable of supporting enough uredia to aid in the dissemination and propagation of wheat leaf rust. Should some of the winter varieties prove to be as nearly susceptible as the spring varieties tested in these studies, they might be considered an aid in the overwintering of *Puccinia triticina*. The only winter variety tested in these experiments was a local strain of Tennessee Winter that proved to be rather strongly resistant to all 6 physiologic forms. Considerable flecking occurred but only a few small uredia developed and the variety could not be considered nearly so favorable a host as some of the spring varieties. Spring seedlings of the more susceptible varieties might aid in the propagation of leaf rust of wheat in some parts of the United States, but the writer has not observed that condition in the Great Plains and adjacent areas.

The data on the reaction of barley varieties to *Puccinia triticina* show that considerable care must be exercised in choosing a variety for use in removing mixtures of that rust from cultures of *P. graminis tritici*. The leaf rust of wheat sporulates on some varieties of barley almost as freely as does the stem rust of wheat.

Considering the difference in the reaction to leaf rust of certain varieties of cultivated barley and of different strains of some of the wild species, it is not surprising that the results obtained by Hassebrauk (6), Waterhouse (10), and others differed. The results obtained in the studies reported herein indicate that with but few exceptions wider differences could be expected from the inoculation of different varieties and strains of barley with a single form, than from the inoculation of a single variety or strain with several physiologic forms.

Among the cultivated barleys the production of uredia was noted in several botanical groups, but it seems to be most marked in the group *Hordeum intermedium cornutum*. Although the number of varieties tested in some of the groups was too small to permit a general statement concerning group reactions, it seems safe to say that pustule formation is not confined to any one group and probably occurs in all groups.

In general there was very good agreement between the results obtained with the cultivated barleys in 1928 and in 1933. One exception worthy of note is the reaction of the two varieties of *Hordeum deficiens steudelii* to form 9 in the two experiments. In 1928 these varieties exhibited a considerable production of uredia, while in 1933 they exhibited mostly flecking with the occurrence of an occasional minute uredium. Such a difference might have been due to differences in greenhouse conditions, but that seems doubtful. It seems more likely that it was due to a slight difference in the rust culture or the seed. Although form 9 was employed in both cases, the culture used in 1933 was of different origin than that used in 1928. It was necessary to obtain a new supply of seed of the varieties used in 1933 and there is a possibility that there was a slight physiologic difference, although the varieties were botanically the same.

SUMMARY

The response of seedlings of certain cultivated varieties and wild species of barley to several physiologic forms of the leaf rust of wheat, *Puccinia triticina*, was studied in the greenhouse.

No cultivated variety or wild species of barley proved to be fully susceptible. However, several economic varieties and some strains of wild species permitted a copious production of small uredia and, therefore, might be considered a factor in the propagation of leaf rust of wheat.

Some barley varieties cannot safely be used to purify cultures of stem rust of wheat because of their tendency to bear reproductive infections of *Puccinia triticina*.

Pustule formation to some extent appeared in nearly all of the botanical groups of cultivated barley but seemed to be most abundant on varieties of *Hordeum intermedium cornutum*.

Greater differences in response to wheat leaf rust were observed when several varieties were inoculated with a single form than when one variety was inoculated with several different physiologic forms.

Among the species of wild barley *Hordeum gussoneanum* C. I. 6067 presented the nearest approach to susceptibility. *H. spontaneum*, *H. maritimum*, *H. bulbosum*, and a strain of *H. murinum* from Idaho exhibited a limited pustule development when inoculated with 3 physiologic forms. *H. nodosum*, *H. jubatum*, *H. pusillum*, and *H. pusillum pubens* were very highly

resistant to all forms, while *H. murinum* and *H. gussoneanum* had highly resistant and moderately resistant strains.

The greatest number of varieties and species exhibiting pustule formation occurred in the 14- and 28-somatic-chromosome groups. *Hordeum nodosum*, with 42 chromosomes, was highly resistant. This is the reverse of the condition in the genus *Triticum* where the greatest number of resistant varieties falls in species having the lower chromosome number, and most of the highly susceptible varieties occur in the vulgare group having 42 somatic chromosomes.

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CONTROL OF SOIL FUNGI BY SOIL FUMIGATION WITH CHLOROPICRIN

G. H. GODFREY¹

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The control of soil-borne organisms injurious to plants constitutes one of the problems in the field of plant pathology not yet satisfactorily met. This paper constitutes the complete report (8) on preliminary studies that have indicated that, by means of an efficient soil fumigant that is both fungicidal and insecticidal in its action, some of the objections inherent in standard methods of soil disinfection may be overcome.

LITERATURE DISCUSSION

For the control of greenhouse-bed and seed-bed organisms injurious to plants, most standard practices involve either a liquid-fungicide, nematicide, or insecticide drench, alone, or combined, or a heat treatment of some sort. The literature is extensive and no effort is made herein to cover it in full. For the control of damping-off organisms in seed beds, various metallic salts of high fungicidal efficiency have been advocated, including those of copper, zinc, and mercury, the latter in both inorganic and organic compounds. Sulphuric acid and acetic acid also have been used for this purpose. All of these require drenching of the soil, and effectiveness in killing the soil fungi extends only so far as the chemical in sufficient concentration penetrates. Guba (14) has demonstrated the efficiency of carbon bisulphide emulsion + formaldehyde in the control of nematodes and *Fusarium* in greenhouses. The cost of this combined treatment is about \$160 per 10,000 square feet or approximately \$700 per acre. Unless the soil is exceptionally well drained, several days or even weeks will be required for elimination of surplus water and chemicals before a crop can be planted. This objection, of course, inheres in the use of any soil-drench treatment.

Bulletins by Newhall (17) and Newhall and Chupp (18) on soil-sterilization methods include the various methods of applying heat. Steam treatments have given excellent results in nematode control and increased yields in tomatoes and other crops, but they involve high expense in the matter

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of installation of equipment, or in labor costs in case installations are not permanent. In Wisconsin costs up to or exceeding \$1000 per acre are common for heat treatments of tobacco and nursery seed beds. Recently developed German machines (1, 23) would appear to give satisfactory steam treatments to soils, but the soil must be handled and moved for the operation. Hot-water treatment carries with it the objection of water-logging the soil, if poorly drained, besides being rather expensive in coal and labor costs.

The use of fumigants for the disinfection of soils has received considerable attention. Hydrocyanic acid gas has been advocated for years for nematode control (5, 16, 25), and Baudys (2) claims for it a certain fungicidal effect. Carbon bisulphide, likewise, has long been used against nematodes (4) and to a certain extent against soil fungi (7), though as a rule it appears to be lacking in effectiveness in the latter respect (14). Chloropicrin has recently (10, and references) been indicated as a decidedly promising chemical for the control of nematodes. In field experiments with pineapples, Godfrey (10) has demonstrated significantly better results with it than with either hydrocyanic acid gas or carbon bisulphide. Roark (20) lists several papers in which the bactericidal and fungicidal effectiveness of chloropicrin is indicated. Cooke (6) attributes a stimulation of sugar-cane growth to the control of a root infecting *Pythium*. Schepetilnikova (22) reports on the use of this chemical in combating flax sickness. Oserkowsky (19) gives the results of laboratory experiments with various volatile chemicals, including chloropicrin, bromopicrin, and others, in which several of the gases under full vapor pressure at room temperature kill the dry sclerotia of *Sclerotium rolfsii*. Chloropicrin was not so effective as bromopicrin.

The advantage of the so-called "partial sterilization" of soil, with a resulting apparent stimulation of plant growth, following treatment with chloropicrin, has been pointed out by Russell (21). Godfrey and his collaborators (12) also have reported a marked stimulation of growth, particularly in tomatoes. They also have emphasized the advantage to be gained from adequate confinement of a fumigant, which is reflected in much enhanced fumigation efficiency. Any fumigant of high vapor pressure rapidly volatilizes and escapes from the soil unless held in by a highly impervious cover. Not only does adequate confinement make for better penetration of the gas into soil clods and the like, as well as to greater depths, but it increases the period of exposure of organisms to the action of the gas; and time is definitely a factor in killing. Godfrey (9) has developed a glue-coated paper that effectively confines chloropicrin gas.

EXPERIMENTS

The experiments here recorded relate solely to soil fumigation with chloropicrin with a view to determining its fungicidal efficiency. A technique is followed similar to that used in nematode-control experiments (12).

Soil. A prepared greenhouse soil consisting of well-screened, loose sandy loam, about 1/6 sand, was used. At the time of the test it contained about 8 per cent moisture on the air-dry-weight basis; well below field capacity, but well above the wilting point. This amounts to about 10 per cent water, by volume. The air-dry soil contained almost exactly 50 per cent air space, as measured by water displacement. The slightly moist soil as used, then, contained air in the proportion of about 40 per cent of the total soil volume. The soil was rendered completely sterile, as shown by culture tests, by autoclaving twice on successive days for 2½ hours at 15 pounds' pressure. This was for the purpose of simplifying reisolation attempts, after fumigation.

Containers. The vessels used for the experiments were 4-gallon glazed stone jars. These were filled to ½ inch from the tops with soil.

Fungus Cultures. *Fusarium* sp.: isolated from gladiolus; supplied by A. W. Dimock of the Division of Plant Pathology of the University of California.

Phytophthora cactorum: isolated from snapdragons; a culture supplied by J. T. Barrett, obtained originally from M. R. Harris of the California State Department of Agriculture. The transfer used was contaminated with bacteria, but the *Phytophthora* was vigorous, and showed abundant cottony growth at the surface.

Rhizoctonia solani: from sugar beet; culture from J. T. Barrett.

Sclerotium rolfsii: from sugar beet. Free sclerotia were used, just as taken from culture tubes of potato-dextrose agar.

Verticillium albo-atrum: from strawberry; obtained from Harold E. Thomas.

Dematophora sp.: isolated from apple roots, transferred to sterilized apple twigs; culture obtained from Harold E. Thomas.

Armillaria mellea: isolated from prune roots, transferred to sterilized apple twigs upon which it grew until the fungus was evident in a stroma-like growth at the distal end from point of inoculation.

The fumigant, chloropicrin, was applied at the rate of 1½ cc. or 2.07 g. per jar; equivalent to approximately 400 pounds per acre-foot of soil. Of the total volume of the jar, 4 gallons, or 15,140 cc., only 40 per cent, or 6056 cc. is air space. According to the Chemical Warfare School, Edgewood Arsenal, New Jersey (24), the maximum possible concentration of gas obtainable at 20° C. and standard pressure is 0.164 grams per liter of air. The air in the jar, then, would be saturated with gas with 6.056×0.164 , or 0.993 grams of chloropicrin. Actually, therefore, an excess of $2.07/0.993$, or 2.1 to 1 was used, disregarding adsorption by soil particles and solution in the soil water. This figure was checked very closely by a rough calculation based upon the proposition that a gram molecule of a gas will occupy 22.4 liters under standard

conditions of temperature and pressure, the gas in this case, of course, being mixed with air at 760 mm., its own vapor pressure being 18.3 mm. at 20° C. The adsorption factor was not calculated. The solubility of chloropicrin in water is 0.14 per cent. With the great amount of water surface exposed in soils, then, a considerable proportion of the available chloropicrin might conceivably be taken up by the soil water. This probably contributes to, rather than detracts from, the effectiveness of the chemical in gas form, for, as shown by Bertrand and Rosenblatt (3) only 5 parts per million in water (which is 1/300 of saturation) stops yeast fermentation entirely. Considering adsorption by the soil and solubility in soil water, then, the soil air in this experiment may or may not have been saturated with chloropicrin gas.

Technique of Manipulation of the Cultures. Small glass cylinders about $\frac{1}{2}$ cm. long and 1 cm. in diameter were filled with loose soil and covered tightly with cheese-cloth tied in place with a string with identifying label attached. These were autoclaved, within Petri dishes. Using aseptic methods, cultures of the various fungi were inserted into the centers of the cells, one to each cell, and a drop of sterile water added to insure the presence of sufficient moisture for fungus growth. The cells, with labels on long strings, were thrust into the jars of soil to about 6 inches depth at points around the periphery. The technique with the root-rotting fungi, *Dematophora* and *Armillaria*, differed from the foregoing in that the inoculated twigs were thrust directly into the soil, without the glass cells. The cultures were allowed to incubate in the soil for 24 hours, before fumigation.

Technique of Fumigation. Using a previously marked pipette for exact measurement, chloropicrin was taken from a dropper bottle and inserted in a hole near the center of the jar to a depth of 3 inches, and covered quickly with soil. Immediately the top of the jar was sealed by gluing over it a circular piece of glue-coated paper cut to size, and sealing it perfectly tightly at the edge with a strip of highly impervious Scotch masking tape, 2 inches wide. There was no opportunity for escape of the gas through any opening at the edges. The only escape that could occur was directly through the paper, and this, as shown by previous tests (9), was possible at only a very slow rate. The test was conducted in duplicate. Duplicate jars with identical arrangement of cultures but without the fumigant, were prepared as controls. Exposure to the fumigant was for a period of 48 hours.

When the covers were removed from fumigated jars, the odor of gas was strong at the surface and in samples of soil lifted from different depths in the jars, showing that penetration of the gas through the soil was efficient.

Culture Attempts. The cultures were lifted by their strings and placed in sterile Petri dishes with loose covers and left there for 24 hours to allow escape of excess chloropicrin possibly still in the cells. It was noted at this time that in the case of the culture of *Rhizoctonia solani*, the period of 24

hours of incubation before fumigation had been sufficient to permit of growth of fungus hyphae, not only throughout the soil in the cell, but into surrounding soil as well, so that surrounding soil was actually lifted with the culture. Transfers were made from bits of the original inocula and surrounding soil to poured plates of potato-dextrose agar.

Results. All fumigated cultures were completely negative for growth up to 10 days after plating, with one exception. The bacterium-contaminated culture of *Phytophthora cactorum* showed growth of the bacteria only. It would appear that this was a resistant, probably spore-bearing soil bacterium. This verifies to that extent the conclusion of Russell (21) and others as to the "partial sterilization" effect of chloropicrin on soils. All control cultures grew normally. Microscopic examination of the profuse hyphal growth of the rhizoetonia culture, that had occurred before fumigation, disclosed a collapsed condition with the protoplasm not evident. Similar hyphae from the control culture were normal. Figure 1 shows Petri-dish cultures from this experiment.

ADDITIONAL TESTS AND OBSERVATIONS

Phytophthora cactorum in Snapdragons. In a large commercial greenhouse near San Leandro, Alameda County, Calif., the phytophthora disease on snapdragons, described by Harris (15), had increased to an alarming degree in the season of 1934 over what had been present the year before. With one variety in particular the mortality was about 25 per cent. In another greenhouse the loss was nearly 100 per cent. By way of a simple experiment to test chloropicrin treatment as a control measure, the writer took soil from beneath wilted plants and placed it in stone jars. One of these was fumigated, as in the foregoing experiment; one was left as a control. The two soils were placed in flats and planted to snapdragon plants pricked from a flat of seedlings. Two weeks after planting, 14 plants of the 56 in the flat of nontreated soil had collapsed and showed typical phytophthora injury at the soil line. At the same time the treated flat showed a 100 per cent vigorous stand. Later, however, one of the plants in this flat failed; still later, 2 or 3 more. Meanwhile, the percentage of failure in the nontreated soil had increased to 50 or more. The infection in the treated flat may have been due to contamination from the other flat, as the two were side by side. Striking efficiency of the chloropicrin fumigation was demonstrated, however.

Verticillium Wilt in Tomatoes. In another experiment, soil from a greenhouse showing abundant verticillium wilt of tomatoes was similarly treated. Tomato plants transplanted from a flat of greenhouse soil in which no *Verticillium* had ever been observed, in the plant pathology greenhouse at the University of California, grew vigorously at first in treated and nontreated soils alike. When the plants were about 15 inches high symptoms of wilting

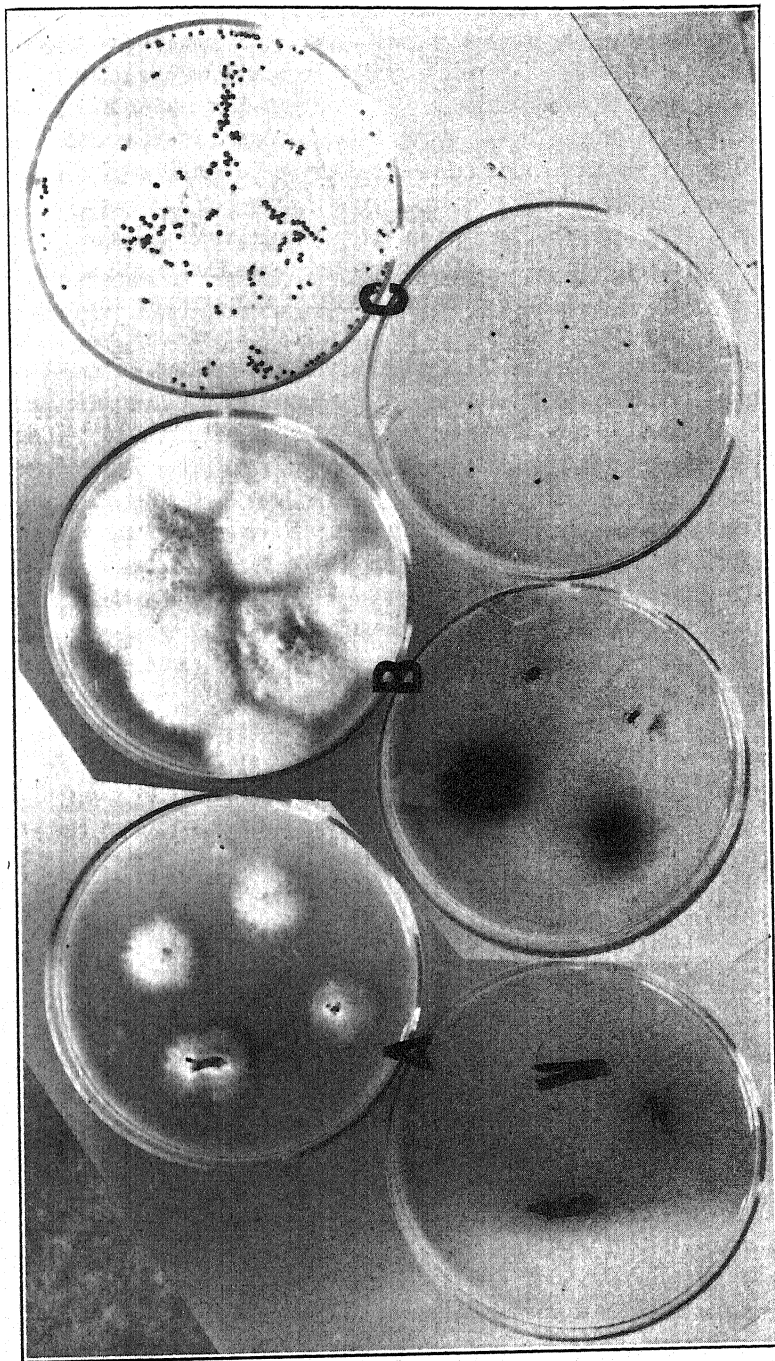


FIG. 1. Results of a fumigation test with chloropicrin. The plates on the top, from nonfumigated fungi; those on the bottom from fumigated ones. A. *Dematophora* sp. on apple twigs. B. *Fusarium* sp. from gladiolus. The dark stain is from the pigment of leaves. C. *Sclerotium rolfsii*. Note complete freedom from growth of sclerotia from the fumigated culture and the abundant reproduction of sclerotia in the nonfumigated culture. All other cultures tested showed results that were just as definitely positive as those illustrated.

occurred in one of the nontreated pots. Shoots cut from the side of this plant and from the other nontreated plant showed typical brown-ring symptoms of verticillium infection. No such symptoms could be found in plants from the treated soil. Subsequently, with the advent of warmer weather in late spring and after new root development had occurred from procumbent stalks, recovery apparently occurred, and, after the dropping of the first wilted leaves, no further wilting occurred up to the time of the last observations. As is well known, with a rise in temperature verticillium-infected plants frequently recover in part. A distinct difference in the size of plants in favor of the treated lot was, however, evident throughout their later growth.

A final experiment, this time in a greenhouse ground-bed, is reported, mainly to record the technique followed. In a house in which verticillium wilt had been observed to a serious degree the previous winter, a plot of soil 6 × 8 ft. in dimensions was set apart by surrounding it with a 6-inch board frame sunk into the ground about 5 inches. The soil, after the usual manure fertilization and deep spading, was treated by means of a Vermorel Injector with chloropicrin at the rate of 400 pounds per acre, at 1 foot intervals. Immediately a glue-coated paper cover was laid over the plot and sealed to the board frame with an efficient paste, leaving no opening whatever. An additional sealing measure consisted in wetting down the soil in a furrow just outside the frame, with a view to preventing the escape of the gas too quickly through the soil. This was kept wet by occasional applications of water during exposure to the fumigant. The cover was left on for 6 days. When it was removed the odor of the gas was still strong, demonstrating high efficiency in gas confinement. Tomato plants were set according to regular practice, a day or two after the cover was removed. Up to the latter part of June, about 3 months after planting, the plants in treated and adjoining nontreated soil had grown equally well and were approximately 6 feet high. Again the warm summer conditions were unfavorable to the development of Verticillium as it had occurred the previous winter. However, several plants in the untreated portion of the house showed definite wilting of some leaves and a few plants were completely collapsed. No plants in the treated soil showed any symptoms of verticillium infection.

Disinfection of Greenhouse Potting Soils. In an attempt to determine the practicability of the disinfection of greenhouse potting soils with chloropicrin, the writer had made some special boxes, as containers of such soils, in which, directly, the fumigation treatment was to be given. The boxes were made air tight and the lid was constructed to fit tightly to permit of sealing the cracks with adhesive tape. When chloropicrin was applied at the standard rate, 2½ cc. per cubic foot, high efficiency was demonstrated in the killing of injurious soil fungi, nematodes, garden centipedes, collembolids, wire-

worms, sowbugs, and the like, and even, to some extent, of weed seeds. The killing of nut-grass, *Cyperus rotundus*, corms by chloropicrin fumigation has already been demonstrated (12).

Chloropicrin for Sterilizing Glassware. The writer has found that ordinary laboratory glassware, such as Petri dishes, can be very efficiently sterilized by stacking them dry in a container that can be tightly closed, such as a coffee can, and putting a few drops of chloropicrin into one of the dishes, then sealing the container tightly with adhesive tape. The quantity of the fumigant used needs theoretically to be only 0.1 cc. to each liter of air space, though doubling or tripling this quantity may be desirable in practical application, in order to insure continued saturation of the atmosphere. A few hours to a day probably is sufficient period of exposure. The dishes must be permitted to stand in an open chamber for 2 or 3 hours for aeration before using for culture work. The equipment for this may prove to be a very satisfactory supplement to the apparatus of a traveling plant pathologist for its obvious advantages of convenience and ease of manipulation.

DISCUSSION

Several factors are to be considered in connection with the use of chloropicrin as a soil fumigant for whatever purpose.

(1) It possesses the property, rare among chemicals, of being simultaneously and without special modification, a fungicide, a nematocide, and an insecticide.

(2) It requires no wetting of the soil. The quantity of water consumed in a drench treatment is no small factor. Guba states that 1000 gallons of liquid are necessary for 1000 square feet of surface.

(3) There is no injurious residue left in the soil. Planting can be done within a day or two after treatment.

(4) Plantings of tomatoes by the writer have never shown any of the toxic effects frequently observed from heat-sterilized soils. Instead, there is often an apparent stimulation of plant growth, such as that reported following "partial sterilization" of soils.

(5) The cost, at the current price of about \$1.00 per pound of chloropicrin, is slightly less than 1 cent per square foot of surface for the fumigant. The cost of a gas-tight paper should not exceed about 1/5 cent per square foot. The total cost of treating 1000 square feet, then, would be:

Chloropicrin	\$10.00
Paper	2.00
Labor, about50

Total \$12.50, or \$125 for 10,000 square feet.

The paper can be used repeatedly, if handled carefully. The cost of chloropierin may be greatly reduced if an extensive application for it in this direction is developed. A recent quotation of \$0.65 per pound in large quantities has been made by one manufacturer. A lower rate of application may prove equally effective. Three hundred pounds per acre gave as good results as higher rates for nematode control (10).

Precautions. For indoor application, such as in greenhouses, it is advisable to use a gas mask. For out-of-door work, with ordinary precautions, this is not necessary. Likewise, for greenhouse work, this material should not be used in a portion of the house occupied in any part by growing plants. It has been found (11) that relatively low concentrations of the gas in the air are injurious to many plants.

The fact of great reduction or complete elimination of beneficial legume bacteria by chloropierin fumigation of the soil (12, p. 1340) should be considered, if a legume is to be planted. With sweet peas, for example, while good control of *Rhizoctonia* is to be expected, the supply of *Rhizobium* should be renewed by seed or soil inoculation if good plant growth be expected.

The rate of diffusion of chloropierin through a continuous layer of water is very low. The soil must not be too wet, as the gas will not diffuse efficiently into soil that lacks open air-spaces between the soil particles. A primary consideration in the use of chloropierin as a soil fumigant, therefore, is that the soil must be in a state of good tilth and on the dry side rather than the wet.

The need for the best possible confinement of the gas in the soil can not be too greatly emphasized. The vapor-pressure gradient between the point of application and the open air is high, 18.3 to 0, and there will be constant diffusion in the direction of escape from the soil unless there is an adequate impermeable cover holding it in. The gradient within the soil is the same at the start and it gradually becomes less with adsorption of the gas by soil particles, solution in the soil water, complete vaporization at the source, and the inevitable escape of gas, until equilibrium is reached. With the release of the gas by removal of the cover the gas is quickly evacuated from the soil. Complete freedom from any break in the confining cover, obviously, is an important consideration. The value of a supplementary barrier of wet soil around a treated plot, to prevent the escape of gas through the soil at the edge, is also evident. Unless the presence of gas in the soil be readily detectable by odor at the end of a period of exposure of perhaps 48 hours, it may be taken for granted that the escape of gas has been too rapid and best efficiency can not be expected.

The Fungicidal Value of Other Fumigants. Whether or not other fumigants have fungicidal value equal to that of chloropierin remains to be determined by specific trial. Hydrocyanic acid gas, carbon bisulphide, tetra-

chlorethane, and ethylene dichloride are to be considered in this connection. Gersdorff's (13) bibliography on the latter chemical contains 4 citations that mention the killing of certain pathogenic bacteria, and yeasts. Its general fungicidal value may, therefore, be worthy of special study.

SUMMARY

Laboratory experiments designed to determine the practical fungicidal value of chloropicrin used as a soil fumigant were conducted with 4-gallon glazed, stone jars of soil. Fungi tested were *Fusarium* sp. from gladiolus, *Phytophthora cactorum* from snapdragons, *Rhizoctonia solani* from sugar beet, *Sclerotium rolsii* from sugar beet, *Verticillium albo-atrum* from strawberry, *Dematophora* sp. from apple roots, and *Armillaria mellea* from prune roots. The cultures were inserted adjacent to the wall of the jar to a depth of 6 inches, then chloropicrin was applied at the rate of $1\frac{1}{4}$ cc. per jar (equivalent to 400 pounds per acre foot) into the center of the jar. Gas-impervious, glue-coated paper was used to seal the jars. Exposure was for a period of 48 hours. Cultural attempts on plates of nutrient agar were completely negative for growth for all fumigated cultures. Controls were all positive. Subsequent small-scale tests with greenhouse soils naturally infested with plant-disease fungi gave good indications, as did also a greenhouse ground-bed test with tomato *Verticillium*. The practicability of disinfecting greenhouse potting soils, in special boxes, was demonstrated. Incidentally, the feasibility of sterilizing laboratory glassware by chloropicrin fumigation was likewise shown.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIF.

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A BACILLUS ISOLATED FROM DISEASED PLANTS OF *AUCUBA JAPONICA* (THUNB.)¹

GEORGE TRAPP, M.A., B.Sc., PH.D.

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INTRODUCTION

During the investigation of a die-back disease of the Japan laurel a bacillus was constantly isolated from stem, leaf, and root lesions of affected plants. Since the organism possesses characters that clearly distinguish it from other forms previously described in bacteriological science and since these differences are of specific rank, the life history of the bacillus has been worked out in some detail.

ISOLATIONS

The organism was obtained as a halo of pure growth, (Fig. 1, A), surrounding sections of diseased leaves, stems, and roots implanted on the sterile surface of plated nutrient agar or gelatin, after thorough surface sterilization of the organs from which the sections were taken. Similar surface-sterile sections cut from corresponding organs of healthy *Aucuba* plants and planted as controls on like nutrient media gave rise to no bacterial growth, thus ruling out the possibility of an epiphytic or commensal origin for the organism.

INOCULATION EXPERIMENTS

Although the presence of the organism internally in the more recently necrosed tissues of *Aucuba* shoots, affected by a blight of the die-back type and accompanied by comparable root lesions, might be taken as an indication of etiological relationship, yet exhaustive attempts to reproduce the symptoms of disease by artificial inoculation failed to establish the pathogenicity of the organism in spite of the adoption of a varied technique and the employment of a diversity of experimental material and conditions involving the inoculation of upwards of 150 plants and cuttings. In all inoculations young and vigorous subcultures from recent isolations were used, the pure cultures of the organism being obtained from different organs and from different plants supplied from a number of distinct localities where the incidence of the disease was observed to be particularly severe.

Under the circumstances it seems advisable to regard the organism as a saprophyte especially adapted to form the vanguard of secondary invaders that dispose of the débris provided by the progress of the primary lethal

¹ Excerpt from thesis approved for the degree of Doctor of Philosophy by the University of Glasgow.

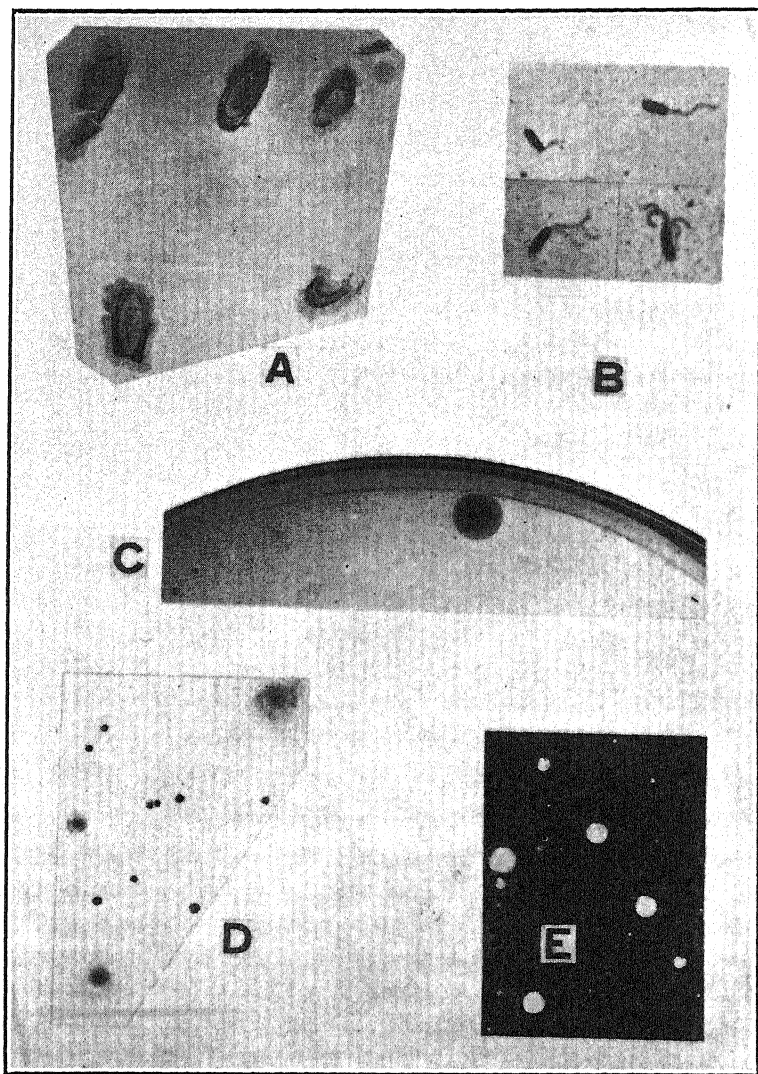


FIG. 1. *Pseudomonas aucubicola*, sp. nov. A. Poured plate isolation of the organism by halos of pure growth from stem sections of diseased aucuba twigs implanted on agar. $\times 0.7$. B. Film preparation of the bacillus from a 2-day-old agar streak culture. The rods possess from 1 to 4 polar flagella. Stained by Kirkpatrick's method. $\times 1,420$. C. Surface and buried colonies of the organism on beef-infusion agar. Photographed by oblique transmitted light from a 3-day-old culture to show homogeneous structure and entire margin of surface colony. $\times 1.6$. D. Surface and buried colonies of the organism on gelatin. The surface colonies show large areas of surrounding liquefaction; the buried colonies are small and their position marked by the occurrence of small pits of liquefaction at the surface of the medium. Age of culture and photography as in C. $\times 1.3$. E. Surface and buried colonies of the organism on gelatin after prolonged subculturing on agar. Note the raised growth of the surface colonies and the absence of liquefaction. Photographed by reflected light from a 3-day-old culture. $\times 0.9$.

agent whose nature has so far evaded elucidation. This tentative conclusion appears the more correct in view of the failure to detect any sign of the organism in appropriately stained microtome sections taken from the junction of healthy and diseased tissues in material that, prior to fixation, showed an actively advancing margin of necrosis.

LIFE HISTORY OF THE AUCUBA BACILLUS

Morphology. The *Aucuba* organism is a short, usually slightly curved, rod with rounded ends, occurring singly or in pairs frequently arranged to form an obtuse angle. Smears from 3 days' old cultures on beef-infusion agar stained with carbol fuchsin give rods varying in length from $0.90\ \mu$ to $1.70\ \mu$ and in diameter from $0.45\ \mu$ to $0.60\ \mu$ and with an average measurement of $0.60\ \mu$ by $1.20\ \mu$. In broth culture the corresponding measurements are $1.70\ \mu$ to $3.40\ \mu$ and $0.60\ \mu$ to $0.85\ \mu$ and an average size of $0.65\ \mu$ by $2.60\ \mu$. No capsules are produced by the organism on agar or in broth when films are stained by Hiss's method. Examined in wet preparations, the organism exhibits a darting or "vibrioid" type of motility and, stained by Kirkpatrick's method² is shown to possess 1 to 4 lophotrichate flagella (Fig. 1, B). No endospores have been observed.

Staining Reactions. The organism is definitely gram-negative and is not acid-fast. It stains well with carbol fuchsin but not so intensely with the aniline dyes. Stained with Loeffler's flagellum stain and with the metachromatic Carbol Thionin refractive granules were sometimes observed at one or both poles of the bacillary body: these granules did not stain as glycogen with iodine.

CULTURAL CHARACTERS³

Beef Infusion-agar Slants. In tubes held at 20°C . the growth along the strokes is moderate to strong, whitish gray in color, slightly convex, translucent at the margin, becoming opaque towards the centre; surface smooth, glistening; margin smooth, with a tendency to flattened undulations; of viscid consistency. A few tiny pits in the surface were frequently observed with the aid of a lens. In old cultures (several months) spiked branched crystals were formed expanding from the surface growth into the medium.

Beef Infusion-agar Plates. On poured plates surface colonies can be detected with the naked eye on the second day and by the sixth day have reached a diameter of 4 mm.; the maximum diameter attained after 10 days, was 11 mm. The surface colonies are at first circular and homogeneous by transmitted light (Fig. 1, C); they are slightly raised, whitish grey in color

² Muir, R., and J. Ritchie. Manual of bacteriology. Ed. 9. 866 pp. Oxford University Press. 1932.

³ Unless otherwise stated the organism was cultivated at 20°C .

with moist glistening surface and smooth, entire margin; later, (8 days onwards), they appear flatter and by reflected light a number of faint slightly raised concentric rings and a few slightly depressed radiate markings are observable; the margin remains entire but is now possessed of major undulations. The buried colonies are small (less than 1 mm. after 6 days) lenticular and brown with an opaque centre and a more translucent regular margin, which becomes granular as the colony ages without increase in size.

Beef-extract (Lemco) Agar Slants and Plates. On this medium the form of the growths obtained was similar to that on beef infusion agar.

Beef-extract (Lemco) Broth. The medium is clouded, most densely towards the surface, after 24 hours. By the second day rim and pellicle formation commenced. The lacy pellicle, though connected with the rim, was easily detached by the slightest agitation and even in undisturbed tubes older cultures showed abundant sediment derived from the dejection of the surface skin.

Potato Slopes. The growth on potato is decidedly brown from the commencement and is fairly abundant though not covering the entire surface of the potato. Growth tends to be somewhat heaped along the strokes; the consistency is viscid; the surface is glistening, smooth and the margin entire but thrown into arcs. Abundant precipitate occurs at the bottom of the water which is otherwise clear. The substance of the potato is discolored throughout to a brown which is less intense than the pigmented growth.

PHYSIOLOGICAL PROPERTIES

Chromogenesis. When grown on beef infusion media the organism produces a light green, water-soluble, extracellular pigment diffusing into the medium of agar slants, which it impregnates completely by the third day. With subsequent growth or aging of the culture, pigmentation is not intensified. No color is formed from media prepared with beef extract (Lemco).

Liquefaction of Gelatin. When the organism is isolated from the host onto gelatin plates and tube cultures inoculated from single colonies, typically liquefying colonies are apparent in the dilution plates by the second day. On the sixth day, the surface colonies, now 4-5 mm. in diameter, are surrounded by a circular area of liquefaction (Fig. 1, D). The colony at this stage, when photographed by oblique transmitted light, consists of a dense white center with zoogloal threads spreading into the liquefied halo, but to the actual margin of liquefaction bacterial growth does not appear to have penetrated. The buried colonies are minute (about 0.5 mm. after 6 days at 20° C.) and brownish opaque with a somewhat diffuse discontinuous margin, the position of the colonies being indicated by depressions at the surface of the medium (Fig. 1, D).

Gelatin Stabs. In stab cultures inoculated immediately from gelatin isolations, liquefaction is rapid; at first napiform, it later becomes saccate and proceeds to completion in 14 days and sometimes even less.

Loss of Power of Liquefaction. On account of the diagnostic and taxonomic importance attached to this property, the following account of alteration in the organism's behavior in this respect, consequent on its prolonged cultivation on agar, may be considered relevant. It was observed that poured gelatin plates inoculated with a culture that had been held on agar through successive transfers gave raised, white, opaque, non-liquefying colonies (Fig. 1, E) of an anomalous (for gelatin) type. In order to determine whether a non-liquefying variant had been segregated or whether the change could be explained on physiological grounds alone, the following experiment was undertaken. A culture of the organism, which for convenience may be designated A, obtained from the liquefied material of a gelatin stab was plated out on gelatin and a typical colony (all the colonies were of the liquefying type) was used to inoculate an agar slope on January 8. After repeated subculturing on agar, poured plates were made from a recent transfer (of March 21) on March 24. After 2 days numerous colonies of the raised circular opaque type were visible on dilutions I and II, but the medium showed no signs of incipient liquefaction. The same procedure was repeated with a parallel culture B obtained from a raised non-liquefying colony on gelatin dilution culture derived from an isolation that had been grown previously on agar for many generations. The inoculum from colony B was first subcultured on agar slants on January 8. After successive transfers it finally was plated out on gelatin on March 24 from a subculture of March 21. By March 26 typical raised colonies had appeared that in growth-form and absence of liquefaction were indistinguishable from those obtained simultaneously and under the same environmental conditions from the culture described above under A. Several days later, however, it was observed that both A and B colonies were sinking slightly into depressions caused by the slow liquefaction of the medium. A final observation on April 12 showed that the gelatin on all A and B plates had been completely liquefied. It is concluded, therefore, that the organism's power to cause liquefaction is, if not permanently destroyed, at least lost temporarily and modified to such an extent by growth on agar as to render diagnosis on this basis uncertain unless the cultural history of an isolation can be specified exactly. Morphologically and physiologically, therefore, except in respect of gelatin, the rapidly liquefying and slowly liquefying forms of the organism are identical.

Gelatin tubes stabbed from cultures isolated on agar and subsequently grown on that medium give entirely different characters from those described previously under gelatin stabs, where the organism was isolated on and inoculated from gelatin. Liquefaction is slow to commence (about the 4th day)

and forms at first just a shallow depression where the surface was punctured and into which the growth sinks. The liquefaction then becomes stratiform and slowly descends into the tube at the rate of 4 mm. after 6 days in a typical case, 13 mm. after 18 days, 25 mm. after 38 days, and after 9 weeks the gelatin was less than half liquefied. On the surface of the solid gelatin below the liquefied zone there was a copious flocculent deposit in a conical heap; rim and reticulate pellicles were formed, the latter being easily precipitable; the liquefied portion of the gelatin was clear and, with beef infusion medium, showed green pigmentation which, however, did not diffuse into the solid gelatin in a manner similar to growth on agar.

Fermentation of Sugars and Alcohols. The following carbohydrates were prepared in concentrations of 1 per cent with peptone water as basic medium: glucose, saccharose, lactose, raffinose, mannite and dulcite. The fermentable solutions were set up with Durham's fermentation tubes and Andrade's indicator. Inoculated from a young agar slant culture and observed every 24 hours up to 4 days and then at intervals of 6 days up till the second month, the tubes at no time showed any indication of the production of acid or gas from the test substances, although in every case the solutions showed evidence of growth with rim and pellicle formation and a moderate amount of sediment. There was no turbidity at the closed ends of the fermentation tubes.

Litmus Milk. In litmus-milk the litmus is reduced without previous change of reaction. The milk at first forms a finely flocculent coagulum and is later completely peptonized. In one example no change was observed till the sixth day, when a rim and sediment had formed and the litmus showed reduction by forming zones with a clear layer commencing at the top. After 22 days a thick creamy layer was present to a depth of 11 mm. from the surface; this was followed by a reduced clear zone of 13 mm., then 13 mm. of incompletely reduced litmus succeeded by a uniform zone extending to the bottom of the tube, which contained an abundant precipitate. After 40 days the litmus was completely reduced and showed no later tendency for the color to return: the milk was completely digested and the whey clear.

Hydrolysis of Starch. Although the organism had no markedly disintegrative effect on potato cylinders after 30 days' growth, the starch of a beef-extract-broth culture containing 1 per cent soluble starch was completely hydrolyzed after 19 days when tested with iodine-potassium iodide solution and compared with noninoculated control tube.

Reduction of Nitrates. Nitrates are not reduced.

Production of Ammonia. A trace of ammonia is produced from beef-extract (Lemco) broth (containing peptone) as detected by inserting into the tubes strips of filter paper previously dipped in Nessler's solution.

Production of Hydrogen Sulphide. Hydrogen sulphide is not produced. There was no blackening following stab inoculations of lead acetate agar.

Voges and Proskauer Reaction. Acetyl-methyl-carbinol is not produced.

Production of Indole. Indole is not produced. Peptone water cultures, 2 days and 24 days old, were tested with Ehrlich's rosindol reagent and a saturated watery solution of potassium persulphate: the sodium nitrite-sulphuric acid method also was applied: both sets of tests gave negative results.

Tolerance of Sodium Chloride. Grown in beef-extract broth containing sodium chloride in concentrations of 1, 2 and 3 per cent and examined after 3 days the organism shows a definite, uniform clouding with rim and pellicle formation in the 1 per cent NaCl medium. In 2 per cent NaCl broth turbidity was uniform only for the uppermost 7 mm.; below, the strands of growth spread down into the medium and finally disappeared, leaving the lower half of the tube unclouded: no rim or pellicle was formed in this case although a few floccules floated at the surface. With 3 per cent NaCl broth there was no turbidity. The same 3 cultures, examined after 20 days, showed growth and the occurrence of sediment in all the tubes, but a comparison of the amount of the deposit formed by the organism in the 3 cases showed that it was inversely proportional to the concentration of NaCl in the respective cultures. Therefore, while growth is not entirely inhibited by a concentration of 3 per cent, the organism is regarded as being sensitive to the presence of sodium chloride.

Relation to Free Oxygen. The organism is an obligatory aërobe. Agar slant tubes, inoculated and placed in a Buchner's tube, the contained atmosphere of which is deprived of its oxygen by a mixture of pyrogallie acid and caustic soda, show no growth, however long anaërobic conditions are maintained. (These anaërobic cultures were retained and examined for signs of growth up to 11 months). No growth occurs along the track of the needle in deep agar and gelatin stab inoculations sealed over, immediately after inoculation, with 10 c.c. of the melted medium. Ordinary stab cultures in deep agar tubes show no growth along the track of the inoculating wire except for the first 2 cm. below the surface, where there was a slight, granular, discontinuous nonspreading growth. The surface of the medium around the stab, however, was completely covered with a thick, smooth, glistening whitish grey growth with adherent rim. The first examination of one such stab culture was made 6 days after inoculation and later after 18 days, but there was no observed increase in the amount or change in the appearance of the scanty subsurface growth. As noted above, there was no clouding in the closed ends of fermentation tubes (Kühn's and Durham's).

Relation to Reaction of Medium. The occurrence of growth on both decidedly acid and decidedly alkaline agar media indicates for the organism a fairly wide range of accommodation to the concentration of hydrogen ions: e.g., abundant growth is obtained on media neutral to phenolphthalein (pH about 8.4). The organism cannot be regarded, therefore, as very sensitive to considerable alterations in the pH of the medium.

Temperature Relations. The optimum temperature for growth lies between 20° and 25° C. Growth occurs at 37° C. but it is very thin, flat and serous, and at this temperature the organism produces no green pigment on beef-infusion media. The maximum temperature is about 40° C. and the thermal death point in the region of 50° C.

Longevity on Artificial Media. Subcultures from liquefied gelatin show that the organism is fairly long-lived (4 to 6 months) on this medium. Cultures on agar slopes, however, appear to be much shorter lived, as a specific example will indicate. A subculture of August 23 when used to inoculate an agar slant on September 24 gave no growth at room temperature, but a loopful of the original culture suspended in beef broth gave a slight turbidity. On placing loopfuls of the broth subculture on an agar slant, typical growth was obtained. The foregoing experience demonstrates the fact that in this instance very few of the bacteria remained viable after a month's cultivation on beef agar.

Technical Description. Rods, slightly curved, with rounded ends, 0.45 to 0.60 by 0.90 to 1.70 μ , occurring singly or in pairs. Motile by 1 to 4 polar flagella. Nonsporiferous: nonencapsulated: nonacid-fast: gram-negative.

Gelatin stab: liquefaction saccate when inoculated from isolations in gelatin: cultivation on agar greatly diminishes this power of liquefaction but it returns slowly to a stratiform type. Liquefied medium greenish.

Agar colonies: circular, whitish grey, slightly raised, smooth, glistening, amorphous and of about 5 mm. diameter by the 6th day.

Agar slant: smooth, glistening, fairly abundant, whitish grey growth of viscid consistency. Beef-infusion media diffused with green water-soluble pigment: no chromogenesis occurs on beef-extract ("Lemco") media.

Broth: turbid, denser at first towards the surface: rim and pellicle formation (after 2nd day): sedimentation occurs later.

Litmus milk: peptonized slowly to completion, with zonal reduction of litmus, in 4 to 5 weeks.

Potato: growth moderate, somewhat raised, brown, viscid, with smooth, glistening surface and entire margin: medium of a less intense brown than pigmented growth.

Indole is not formed.

Nitrates are not reduced.

No acid or gas from glucose, saccharose, lactose, raffinose, mannite and dulcitol (in peptone-water medium).

Starch hydrolysis moderate.

Ammonia production doubtful.

Acetyl-methyl-carbinol is not produced.

Hydrogen sulphide is not produced.

Sodium chloride, in concentrations of 1, 2 and 3 per cent in broth caused

retardation of growth proportional to the concentration of salt present, *i.e.*, sensitivity to the presence of sodium chloride is indicated.

Aërobic, obligatory.

Optimum temperature 20° to 25° C. Thermal death point about 50° C.

Habitat: isolated from the recently decayed tissues of roots, stems and leaves of the Japan laurel.

CONCLUSION

A careful comparison of the characters of nearly related species of *Pseudomonas*, as recorded in the Fourth Edition of Bergey's Manual,⁴ with those of the organism described in the present work showed that the latter possessed specific differentia. *Pseudomonas aucubicola*, sp. nov., is therefore offered as the name of the organism. It may also be pointed out that no previous mention has been made of *Aucuba* as a bacterial host plant (*vide* Elliott's list of Bacterial Plant Pathogens [and saprophytes]).⁵

SUMMARY

A motile bacillus, specifically characterized as *Pseudomonas aucubicola*, sp. nov., was consistently isolated in pure culture from the more recently necrosed portions of stem, leaf and root lesions on plants of *Aucuba japonica* Thunb., affected by blight symptomatically distinguished by local die-back of twigs and root terminals and in some cases by acronecrosis of individual apical leaves.

The morphological, cultural, and physiological aspects of the organism's life history are described in detail.

In view of the lack of positive result from carefully planned and comprehensive inoculation experiments and the failure to detect its presence in suitably stained histological preparations of tissue in active necrosis, the organism is provisionally regarded as a saprophyte of special habitat.

ACKNOWLEDGMENTS

The investigation was carried out during the tenure of a grant from the Department of Scientific and Industrial Research. I am also indebted to Dr. S. G. Jones, Lecturer in Mycology at Glasgow University, for suggesting the research and supervising its progress, and to Professor C. H. Browning, of the Pathology Department, Glasgow University, through whose courtesy facilities for part of the work were provided at the Bacteriological Laboratories of the University and Western Infirmary.

BOTANY DEPARTMENT,
UNIVERSITY OF GLASGOW,
GLASGOW, SCOTLAND.

⁴ Bergey, D. H. Manual of determinative bacteriology. Ed. 4. London. 1934.

⁵ Elliott, Charlotte. Manual of bacterial plant pathogens. London. 1930.

STUDIES ON THE ORIGIN OF YELLOW-MOSAIC VIRUSES^{1,2}

JAMES H. JENSEN

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INTRODUCTION

Evidence that has been accumulating during the past few years indicates clearly that closely related strains of tobacco-mosaic virus exist in nature. This is also true of cucumber-mosaic virus and latent-mosaic virus of potato. Whether these viruses have long existed as they are found today, or whether new strains are continually arising, is a question that has received some consideration in the literature but concerning which there is still much need for further study. This question is of importance, not only from a theoretical viewpoint regarding the nature of viruses, but also from a practical standpoint, since plants affected by attenuated or mild-disease-producing strains of certain viruses acquire immunity from more severe strains of the same viruses. The occurrence of yellow-mosaic viruses in irregular-shape bright yellow spots that appear occasionally on mottled leaves of tobacco plants infected with tobacco-mosaic virus seems to offer a means for studying further the question of origin of strains of tobacco-mosaic virus.

The evidence obtained previously (4) regarding the origin of yellow-mosaic viruses may be summarized briefly as follows: The viruses were isolated from plants infected with tobacco-mosaic virus that had been repeatedly transferred from single necrotic lesions on leaves of *Nicotiana glutinosa* L. plants. It was believed that repeated passage of tobacco-mosaic virus through single necrotic lesions would serve to free it from all traces of yellow-mosaic virus that might have been present originally. It was concluded from these experiments that yellow-mosaic viruses arise during multiplication of tobacco-mosaic virus in infected plants.

It is the purpose of this paper to report the results of further experiments designed to test the validity of this conclusion. These additional experiments were concerned with the questions: whether bright yellow spots would appear on tobacco plants inoculated with samples of tobacco-mosaic virus believed to be derived from single infectious units; whether the yellow-

¹ The experimental work reported here was carried out in the laboratories and green-houses of The Rockefeller Institute for Medical Research at Princeton, New Jersey, and of the Department of Plant Physiology at the University of Wisconsin. The writer acknowledges with pleasure his indebtedness to Dr. B. M. Duggar and Dr. L. O. Kunkel for criticism and guidance throughout the course of this investigation.

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mosaic viruses isolated from such bright yellow spots were strains of tobacco-mosaic virus; and whether these yellow-mosaic viruses would, in turn, give rise to other viruses.

MATERIALS AND METHODS

The tobacco-mosaic virus used was derived from virus contained in a single necrotic lesion on a leaf of *Nicotiana langsdorffii* Schrank. The lesion resulted from a single pin-puncture inoculation of tobacco virus 1 (5), a sample of which was kindly supplied by James Johnson, of the University of Wisconsin.

The experimental attempts to obtain single infectious units of virus fall roughly into two types: (A) those dependent entirely on mechanical action, *i.e.*, high dilution and single pin-puncture inoculation; (B) those in which the virus remaining after some special treatment was inoculated into leaves of *Nicotiana langsdorffii* plants and subsequently transferred from the necrotic lesions produced. The lesions on *N. langsdorffii* are large (Fig. 1) and virus is relatively easily recovered from them. It is believed that single necrotic lesions result, in most cases, from inoculation with single infectious units of virus (Kunkel 6).

The tobacco plants used were *Nicotiana tabacum* L. var. Turkish. Unless otherwise specified, all plants were grown in fertilized soil in 4-inch or 6-inch unglazed clay pots or in shallow wooden flats. The greenhouse temperature during the course of experiments reported varied from about 68° F. to about 90° F., with an average temperature of about 72° F.

The rubbing method of inoculation (Holmes 3) was used in all gross transfers of virus. Other special methods of inoculation used have been described previously (4).

YELLOW-MOSAIC VIRUSES IN PLANTS INOCULATED WITH VIRUS BELIEVED TO HAVE BEEN DERIVED FROM SINGLE INFECTIONOUS UNITS

Attempts were made to obtain further information regarding the origin of yellow-mosaic viruses through the use of tobacco plants inoculated with tobacco-mosaic virus samples believed to be derived from single infectious units. A record was kept of the yellow spots that appeared on these plants and of the yellow-mosaic viruses isolated from the spots. The attempts to obtain single infectious units of virus and the results of inoculations with samples of virus derived from them are described in the following experiments.

Isolation by Means of High Dilution. Expressed juice from diseased tobacco plants was diluted 1:10,000,000 with distilled water. A sterilized cheesecloth pad was then saturated with the diluted juice and rubbed over one leaf on each of 1247 healthy young Turkish tobacco plants. Within 14

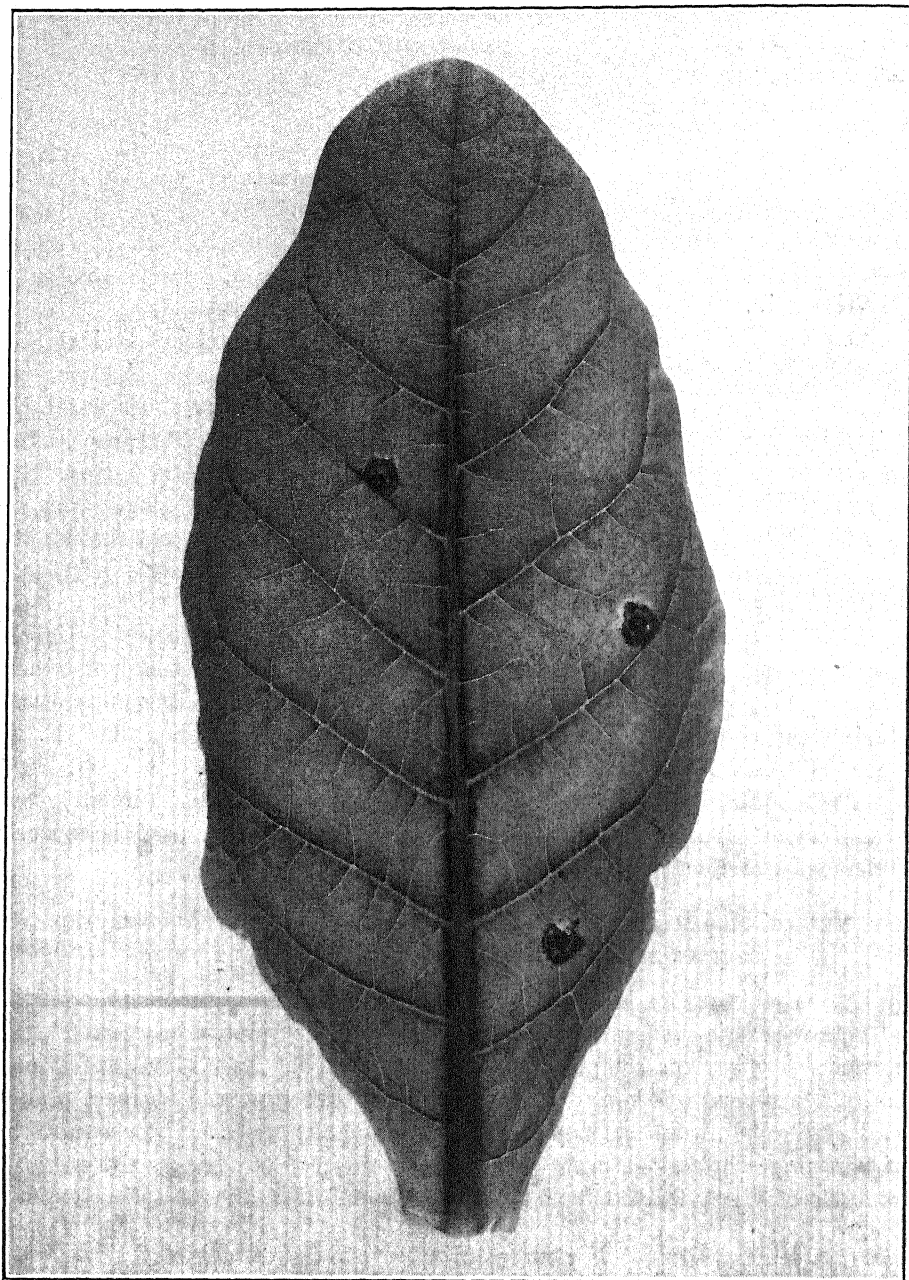


FIG. 1. Necrotic primary lesions on a leaf of *Nicotiana langsdorffii*. Isolated necrotic lesions are believed to be the result of inoculation with single infectious units.

days after inoculation, a total of 24 plants showed typical symptoms of tobacco mosaic infection. Eight days after inoculation, bright yellow spots appeared on some of the infected plants. Within 40 days after inoculation, all diseased plants showed one or more of these spots. Single pin-puncture isolations were made from bright yellow spots on 2 different plants, and 2 unlike yellow-mosaic viruses were recovered. They are of the "slow-moving" type; in tobacco they move from the point of inoculation so slowly that they rarely reach the tip leaves. They usually produce blotch-like yellow primary lesions only. Both viruses are difficult to transmit by simple rubbing methods when in undiluted juice, and even more difficult to transmit after dilution.

The development of one or more typical bright yellow spots on leaves of every infected plant and the demonstration that yellow-mosaic viruses were present in some of these spots show that such viruses occur in tobacco plants inoculated with juice containing tobacco-mosaic virus diluted 1:10,000,000.

Isolation by Means of Single Pin-puncture Inoculation. From a diseased leaf that showed no bright yellow spots, single pin-puncture transfers were made to small tip leaves of 100 healthy young tobacco plants. Within 7 days, 55 of these inoculated plants showed typical clearing-of-veins symptoms of tobacco-mosaic infection. Nine days after inoculation, the first bright yellow spot appeared. Within 44 days, one or more yellow spots had appeared on every diseased plant. Yellow-mosaic viruses were isolated from 4 bright yellow spots in this set of plants. The experiment furnished evidence that yellow-mosaic viruses occur in tobacco plants infected with tobacco-mosaic virus by single pin-puncture inoculation.

Isolation by Means of Ultrafiltration. Ten cc. of frozen juice of diseased tobacco plants were added to 35 cc. of 0.1 M Na_2HPO_4 solution and passed through a Seitz filter under pressure. Ten cc. of the filtrate were then passed through a collodion membrane, the average pore size of which, as determined by rate of water flow, was about 90 m μ . According to experiments reported by Thornberry (15), this pore size in collodion membranes permitted the passage of a very limited number of tobacco-mosaic particles. The collodion membrane filtrate was rubbed over 3 leaves of each of 16 *Nicotiana langsdorffii* plants. A total of 10 necrotic lesions appeared on the 48 leaves rubbed. Untreated, expressed juice from diseased plants rubbed over the leaves of 3 control plants produced over 1000 lesions per inoculated leaf, whereas untreated healthy juice rubbed over the same number of leaves produced no necrotic lesions. Virus from each of the 10 necrotic lesions when transferred to a healthy young tobacco plant produced typical symptoms of tobacco-mosaic infection. Within 40 days after inoculation, one or more bright yellow spots appeared on each plant. Yellow-mosaic virus was isolated from 3 of these spots.

This experiment demonstrated that yellow-mosaic viruses were present in tobacco plants inoculated with samples of tobacco-mosaic virus that had passed membranes with pore sizes approaching the limit that permits virus particles to pass.

Isolation by Treatment with Chemicals. Further attempts to obtain samples of tobacco-mosaic virus believed derived from single infectious units were made by testing partially inactivated samples of virus that had been treated with certain chemicals (chloramine-T, lead acetate, silver nitrate, and potassium permanganate) known to act deleteriously (14). In these experiments the chemicals were made up in solutions double the strength indicated in table 1. The treatments were carried out by mixing equal

TABLE 1.—*Number of necrotic lesions obtained on 9 leaves of Nicotiana langsdorffii rubbed with diseased juice treated with various chemicals*

Substance added	Final concentration	No. lesions	Final concentration	No. lesions
Chloramine-T	2% ^a	420 ^b	4%	13 ^c
Lead acetate	1%	103	2%	90 ^c
Silver nitrate	4%	10 ^c	8%	0
Potassium permanganate ..	1%	455	2%	70 ^c

^a Obtained by mixing equal volumes of solution, double the concentration indicated, and clarified diseased juice.

^b 7500 necrotic lesions were produced when an equal number of leaves were rubbed with untreated diseased juice diluted in 1 part water.

^c Virus from 10 single necrotic lesions was transferred to 10 healthy young tobacco plants.

volumes of the solutions and expressed diseased tobacco juice, partially clarified by freezing and centrifugalization. After standing for a period of one hour, the deleterious materials were removed from the solutions by dialysis and the remaining treated juice rubbed over leaves of *Nicotiana langsdorffii* plants. In each instance, as shown by table 1, the number of necrotic lesions obtained by inoculation with treated juice was much smaller than that obtained by inoculation with nontreated juice. Virus from 10 single necrotic lesions obtained by inoculation with samples of juice treated with each of the 4 chemicals was transferred to 10 tobacco plants. Within 50 days after inoculation, one or more bright yellow spots had appeared in every tobacco plant inoculated with virus from a single necrotic lesion. Single pin-puncture inoculations demonstrated that virus of yellow mosaic was present in 11 of the bright yellow spots tested.

Expressed juice of diseased tobacco plants was adjusted to pH 1.0 and pH 11.5 by the addition of 2 N HCl and 2 N NaOH, respectively. Stanley (13) has described the rate of virus inactivation at these hydrogen-ion concentrations. Samples of treated juice, taken at various intervals after the beginning of the treatment, were neutralized and then rubbed over leaves of *Nicotiana langsdorffii* plants. Table 2 presents the number of necrotic

TABLE 2.—*Number of necrotic lesions obtained on 9 leaves of Nicotiana langsdorffii rubbed with neutralized samples of 1:3 diluted diseased tobacco juice which had been held for various lengths of time at one or the other of two hydrogen-ion levels*

Diseased juice adjusted to pH 1.0 by addition of 2 N HCl					
No. hours treated	$\frac{1}{2}$	24	48	54	
No. lesions obtained	1410	885	230	120 ^a	
Diseased juice adjusted to pH 11.5 by addition of 2 N NaOH					
No. minutes treated	1	15	25	35	45
No. lesions obtained	870	241	176	38	26 ^a

^a Virus from 10 single necrotic lesions was transferred to 10 healthy young tobacco plants.

lesions obtained in the various inoculations. Virus from 10 single necrotic lesions that resulted from infection with the samples of partially inactivated inoculum was transferred to young tobacco plants. In all instances bright yellow spots appeared on leaves of the infected tobacco plants within 50 days after inoculation. It was demonstrated by means of single pin-puncture inoculations that virus of yellow mosaic was present in at least 7 of the bright yellow spots.

Expressed juice of frozen diseased tobacco plants was diluted 1:3 with water, adjusted to pH 3, treated with commercial pepsin at the rate of 10 mg. per cc., and incubated at 35° C. Stanley (12) has reported the proteolytic action of this enzyme on tobacco-mosaic virus. At various intervals after the beginning of treatment, samples of treated virus were neutralized and inoculated into leaves of *Nicotiana langsdorffii*. Table 3 shows the re-

TABLE 3.—*Number of necrotic lesions obtained on 9 leaves of Nicotiana langsdorffii leaves rubbed with diseased tobacco juice which had been treated with commercial pepsin for various lengths of time*

No. of hours treated	$\frac{1}{2}$	24	48	72	96
No. of lesions obtained	2500	430	290	140	38 ^a

^a Virus from 10 single necrotic lesions was transferred to 10 healthy young tobacco plants.

duction in virus concentration, as measured by the numbers of necrotic lesions produced, due to digestion by the enzyme. Virus from 10 single necrotic lesions that resulted from infection with the 96-hour treated juice was transferred to young tobacco plants. Yellow spots appeared in each inoculated tobacco plant. By means of single pin-puncture inoculation, yellow-mosaic virus was shown to be present in at least 3 bright yellow spots.

In the preceding pages a number of experiments have been described in which attempts were made to obtain single infectious units of tobacco-mosaic virus. It is believed that numerous samples of virus derived from single infectious units were obtained and tested. In all instances tobacco plants infected with such samples produced, in addition to the ordinary symptoms of tobacco mosaic, bright yellow spots that contained yellow-mosaic viruses. The evidence obtained indicates that yellow-mosaic viruses arise in tobacco plants infected with tobacco-mosaic virus believed to have been derived from single infectious units.

SUDDEN APPEARANCE OF NEW VIRUSES IN PLANTS INOCULATED WITH YELLOW-MOSAIC VIRUSES

It was observed that in sets of plants inoculated with any one of the various yellow-mosaic viruses, occasional plants suddenly exhibited symptoms different from other plants in the same set. Most frequently these sudden changes in symptoms were characterized by the appearance of green mottling of the tip leaves. Transfers of virus from such leaves produced symptoms in tobacco that resembled those produced by ordinary tobacco-mosaic virus infection. In a number of other instances a systemic yellow-mosaic infection suddenly appeared in plants inoculated with a slow-moving virus, which rarely produced symptoms on any leaf other than that inoculated. Owing to the fact that no control plants ever showed such symptoms of infection, it is believed that in each case the unusual symptoms were the result of infection with a new virus that suddenly arose from the virus originally transferred to the plant.

A number of experiments were designed to test further the possibility that these variations in symptoms might be due to contaminations. The general plan of the experiments was to inoculate 20 plants with a yellow-mosaic virus that was believed to be pure and free from other yellow-mosaic viruses. The 20 inoculated plants were surrounded by 20 control plants, the leaves of which were rubbed with healthy tobacco juice. When a slow-moving virus was transferred to the test plants, ordinarily no symptoms appeared on any leaves other than those inoculated, even though plants were held as long as 2 months. It is most improbable that any virus of the green-mottling type was continually present in these cultures. In several experiments, however, after plants inoculated with a slow-moving virus had been

held on the bench for 30 or 40 days, one or two plants suddenly exhibited mottling of the tip leaves. Green-mottling viruses were usually obtained upon sub-transfers from these leaves. Occasionally, yellow-mottling viruses were recovered from plants inoculated with slow-moving viruses. In all experiments the control plants showed no symptoms, nor was virus recovered from any of them, although repeated trials were made.

Other experiments carried out with yellow-mosaic viruses that produce systemic infection gave similar results, but, because of the type of infection, the results were not so striking. Green-mottling viruses were occasionally obtained from the tip leaves of plants infected with virus that had been repeatedly transferred by single pin-puncture inoculations, although all other plants of the sets were infected with only the yellow-mosaic virus and showed no divergent symptoms.

In addition to the isolated instances in which unusual symptoms developed in inoculated plants, another type of variation was observed. Now and then irregular-shape bright yellow spots appeared on leaves of plants mottled with yellow-mosaic virus. From two such spots viruses have been isolated that were different from those originally introduced into the plants.

It is concluded from these experiments that yellow-mosaic viruses originally isolated from yellow spots on plants infected with tobacco-mosaic virus sometimes give rise to other viruses.

DIFFERENCES IN YELLOW-MOSAIC VIRUSES AND THEIR RELATION TO TOBACCO-MOSAIC VIRUS

During the course of these investigations, 51 samples of yellow-mosaic viruses have been isolated by means of single pin-puncture transfers from bright yellow spots on plants infected with tobacco-mosaic virus. The various yellow-mosaic viruses show great differences in (A) the intensity of the yellow symptoms, the amount of distortion, and the amount of necrosis produced in leaves of tobacco, (B) the intensity of yellow symptoms and the amount of stunting produced in tomato, (C) the type of infection, *i.e.*, necrotic or chlorotic, produced in *Nicotiana sylvestris* Speg. and Comes, (D) the relative speed of movement from point of inoculation, and (E) relative infectivity. On the basis of the above-mentioned symptom variations, it can be stated definitely that many, if not all, of the viruses isolated are different from tobacco-mosaic virus and from each other. Some of them have been transferred continuously over a period of more than 2 years and during this time have retained their original characteristics. With the exception of certain variations mentioned in the previous section, all viruses have retained their individual characteristics when repeatedly transferred or when held in dried leaves or frozen juice.

Although many, if not all, of the yellow-mosaic viruses isolated are different from each other, there are several lines of evidence demonstrating that

all of them are strains of tobacco-mosaic virus. The 51 yellow-mosaic viruses were isolated from leaves of plants infected with tobacco-mosaic virus derived from virus originally contained in two single necrotic lesions on leaves of *Nicotiana langsdorffii*. Chester (1), using both the precipitin and complement-fixation reactions, has demonstrated that about 20 of these viruses are serologically indistinguishable from tobacco-mosaic virus. All yellow-mosaic viruses produce infection in tobacco, tomato and *N. sylvestris*, and all produce necrotic local lesions on leaves of *N. glutinosa*. Many of the viruses have been kept in frozen juice or in dried leaf tissue for 2 years. Other viruses, isolated more recently, have not been tested in this connection. None of the yellow-mosaic viruses, with the possible exception of those of the "slow-moving" type, are inactivated by heating for 10 minutes at 80° C. All yellow-mosaic viruses are transmissible by mechanical methods. These properties and host reactions are characteristic of viruses belonging to the tobacco-mosaic virus group.

The evidence presented above indicates that many different yellow-mosaic viruses have been isolated from bright yellow spots on leaves of tobacco plants inoculated with virus of typical tobacco mosaic, and that all of these viruses are strains of tobacco-mosaic virus.

DISCUSSION

Conclusive proof that variant strains of viruses arise from the tobacco-mosaic virus will be difficult to obtain until it is possible to separate with certainty single units of virus. Nevertheless, several lines of experimental evidence have been obtained, which, taken individually and collectively, strongly indicate that yellow-mosaic virus strains arise suddenly from the ordinary green tobacco-mosaic virus.

The most important evidence that variant strains arise from tobacco-mosaic virus is found in the fact that so many different strains showing some characteristics of tobacco-mosaic virus were isolated from plants infected with the virus of tobacco mosaic. Including experiments, presented in another report (4), 51 isolations of yellow-mosaic viruses have been made. Although only preliminary studies have been conducted on them, it is clear that many, if not all, differ from each other and from tobacco-mosaic virus. The strains have all been isolated from plants infected with virus originally present in one or the other of two single necrotic lesions. It seems altogether unlikely that virus of so many strains of yellow mosaic, in addition to the tobacco-mosaic virus, were present in each of these single necrotic lesions. Furthermore, the first tobacco leaves inoculated with virus from the single necrotic lesions developed no primary yellow lesions such as would have developed had the slightest trace of yellow-mosaic virus been intentionally added. The first indication that virus of yellow mosaic was present came

when a bright yellow spot appeared on a systemically diseased leaf of an inoculated tobacco plant. Some of the viruses isolated were not obtained until more than a year after virus in the single necrotic lesions was transferred, and, therefore, did not appear until many sub-transfers of the original virus had been made.

Yellow-mosaic viruses, difficult to transmit with ordinary rubbing methods of inoculation, were isolated from plants under conditions that practically precluded their having been present in the virus transmitted to the plants. In one experiment two different viruses, neither of which can be transmitted when diluted more than 1:100, were isolated from plants infected with tobacco-mosaic virus diluted 1:10,000,000.

In view of the evidence presented, it seems most improbable that the yellow-mosaic viruses are carried along in every single infectious unit of tobacco-mosaic virus. If viruses of such strains are carried along they must be linked in some manner with tobacco-mosaic virus and this bond must be broken when yellow-mosaic viruses appear in bright yellow spots.

It is of interest to note that, although viruses have been isolated that produce a wide range of symptoms, all are definitely related to tobacco-mosaic virus. In no instance have viruses been obtained having the properties of cucumber-mosaic virus, tobacco ring-spot virus, or any other virus unrelated to that of tobacco mosaic.

That the yellow-mosaic viruses, isolated from plants infected with virus of tobacco mosaic, in turn give rise to other yellow-mottling viruses or to green-mottling viruses similar to that of tobacco mosaic, is further demonstration that one virus may arise from a related virus. In such a demonstration the interrelationship of the different viruses of the tobacco-mosaic group is indicated and evidence of the variability of these viruses is strikingly brought out.

Regarding the origin of yellow tobacco-mosaic virus, McKinney (7) in 1929 stated that "it seems entirely possible that viruses may be altered locally in a plant, thus producing mutations, to use this term in its broadest meaning." Two years later in an abstract (8), McKinney stated that he considered the common mosaic of tobacco to be a "virus complex." Without presenting details of his experiments (9) he reported that he was unable to completely eliminate traces of yellow-mosaic virus in tobacco-mosaic virus, even by dilution and treatment with alcohol, acetone, acids, alkalis, high temperatures, ultra-violet radiation, centrifugalization, filtration, and inoculation to different species. McKinney (10) regarded the yellow and green mosaic of wheat as due to a mixture of two viruses, separable by repeated subinoculation. Jensen in 1933 (4) presented evidence from which he concluded that yellow tobacco-mosaic viruses arise during multiplication of green tobacco-mosaic virus in infected plants. Price (11), working with

cucumber-mosaic virus, brought evidence indicating that new strains of virus arise by mutation or some similar process in tobacco plants having the cucumber-mosaic disease. Hoggan (2) also obtained evidence of strain variation in cucumber-mosaic viruses.

Whether the new strains of virus that appear in infected plants should be designated as variant strains, mutant strains, viruses causing mutant symptoms, or by some other term is a question which cannot be settled definitely on the basis of our present knowledge.

SUMMARY

Experimental results were obtained yielding further evidence that yellow-mosaic viruses arise in plants infected with tobacco-mosaic virus. Attempts were made to obtain single infectious units of virus by means of high dilution, single pin-puncture inoculations, ultrafiltration, and chemical treatments. It is believed that many single infectious units of virus were obtained. All tobacco plants inoculated with virus derived from such units produced, in addition to the usual symptoms of tobacco-mosaic infection, bright yellow spots from which yellow-mosaic viruses were isolated.

In sets of tobacco plants infected with any one of the strains of yellow-mosaic viruses, occasional plants produced symptoms that were different from those produced by other plants of the same set. Sub-transfers of virus from tip leaves of plants showing unusual symptoms resulted in the isolation of virus unlike that originally introduced. It is believed that new viruses occasionally arise in plants inoculated with any of the yellow-mosaic virus strains.

Fifty-one isolations of yellow-mosaic viruses have been made. On the basis of variations in symptoms, rates of movement, infectivity, etc., it is concluded that many, if not all, of these viruses are different from each other. On the other hand, certain properties, host reactions, and serological relationships demonstrate that the yellow-mosaic viruses are strains of tobacco-mosaic virus.

The evidence strongly indicates that new strains of tobacco-mosaic virus arise suddenly in infected plants by some process apparently similar to that of mutation.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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SEEDLING CULTURE IN SAND TO PREVENT DAMPING OFF

A. A. DUNLAP

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Pure sand with inorganic salts added in solution has been found to be a suitable medium for the culture of many species of seedlings. This method eliminates most of the damping-off of seedlings so prevalent in soil culture. Furthermore, the growing of seedlings in sand makes possible certain desirable cultural practices.

It has been known that damping-off of seedlings is less apt to occur in light sandy soils than in those containing large amounts of organic matter. Sand also has been used to cover the soil surface in attempting to reduce the loss of seedlings through fungous attacks. However, by growing seedlings in pure sand, a medium is employed that, in the beginning, is entirely incapable of supporting the growth of most fungi. The only sources of organic material, necessary for fungous growth in such a culture, are later introduced with the seed material, or are formed by the growth of chlorophyll-bearing plants.

MATERIALS AND METHODS

In this investigation the sand used was a brown sea sand from Long Island. It had a water-holding capacity of about 24 per cent. In regard to particle size, 30 per cent of the particles were less than 0.4 mm., 40 per cent were from 0.4 mm. to 0.8 mm., and the remaining 30 per cent were larger than 0.8 mm. in diameter. Good results also were obtained with sand of other types; however, a few kinds of sand from certain inland sources have been found unsatisfactory.

For the most part the cultures were grown in glazed crocks, 17 cm. in diameter, 10 cm. in depth, with a 1-cm. drainage hole in the bottom. Certain seedlings have been successfully grown in 2 or 3 centimeters of sand in water-tight containers, as well as in ordinary wooden flats. The cultures have been kept in the greenhouse except for certain trial cultures in cold frames and out-of-doors, which have shown results similar to those in the greenhouse.

The sand was washed in hot water (60° to 90° C.) until all sediment was removed, before filling the crocks. To each crock of sand was added 20 cc. of a 0.05 per cent solution of soluble FePO_4 by sprinkling. In the same manner 70 cc. of a nutrient solution with the following composition was added: $\text{Ca}(\text{NO}_3)_2$ — 2 g.; KNO_3 — 4 g.; $\text{CaH}_4(\text{PO}_4)_2$ — 1.2 g.; MgSO_4 — 1.2 g.; water — 1000 cc. Commercial grades of chemicals were used. A solution of higher than usual concentration was used to provide

the culture with sufficient nutrient to last through the seedling period with the usual watering and thereby make post-emergence fertilization unnecessary. Later applications of a more dilute solution were made when prolonged growth of the seedlings was desired. Nutrient solutions of this type gave pH readings of from 4.4 to 4.6, but, after addition to the sand, this reading was found eventually to be much higher. Experiments with other solutions in sand cultures have indicated that for most seedlings the type of nutrient solution is relatively unimportant as long as there are suitable amounts of nitrogen and potassium present. It appears possible for practical purposes that a 2-salt solution may be satisfactory in growing many kinds of plants through the seedling stage.

A definite number of seeds, depending upon the species, were placed in each crock after the addition of the nutrient solution. The seeds were covered with a layer of sand and the surface of the culture was then watered. Certain seeds that apparently require more oxygen in germination were covered with coarse sand or with sand mixed with such materials as asbestos or granulated charcoal. Enough sand was used in covering to hold the seedlings in place upon emergence, but not deep enough to seriously retard germination. Until the seedlings were ready for transplanting into soil, the only further attention necessary was the watering of the culture.

Soil cultures were similarly prepared and kept with the sand cultures under the same conditions and received the same amounts of water. The soil used in this work was for the most part a composted greenhouse soil containing light sandy loam, rotted manure, and leaf mold. The *Rhizoctonia* organism causing damping-off was present in the soil and a fungus of the *Pythium* type was found less frequently. Besides growing cultures of seedlings in the nontreated soil, other soil cultures were maintained with some of the more common soil and seed treatments. Some of the crocks of soil were autoclaved at 15 lbs. pressure for 2 hours, while others were treated with 20 g. of a 6 per cent formaldehyde dust. Certain seed treatments, such as cuprous oxide, Semesan, and zinc oxide also were used with nontreated soil and the results compared with the sand cultures.

EXPERIMENTAL RESULTS

After the emergence of the first seedlings, each culture was examined daily for seedlings showing damping-off. The diseased seedlings were removed and the number recorded. When the seedlings had reached an ample size and were sufficiently mature for transplanting, they were removed from the substrate, counted, and weighed.

In table 1 are given the results of controlling damping-off by means of sand culture, expressed in terms of the number of healthy seedlings that had survived up to the time of transplanting, as compared with similar

TABLE 1.—*Comparison of seedling production in sand and in nontreated soil*

Species	Number of seeds per culture ^a	Age of culture in days	Number of healthy seedlings	
			Soil	Sand
Aquilegia	150	40	48	85
Beet	200	20	65	282
Cabbage	250	15	177	203
Celery	500	40	69	458
Cosmos	150	12	48	105
Delphinium	200	25	27	65
Gypsophila	500	20	87	404
Lettuce	300	15	51	267
Pepper	150	25	78	110
Pine (white)	100	50	9	34
Salvia	200	25	56	95
Spinach	250	20	43	127
Spruce	200	50	10	105
Tomato	150	20	96	118
Zinnia	150	15	41	85

^a This number of seeds for each species was used in each culture throughout this work, either in sand or in soil.

culture in nontreated soil. This table also shows the number of seeds planted in each culture and the number of days from the time the seeds were planted until the final data upon the cultures were taken. These results include the averages of a large number of individual cultures grown at different seasons over a period of 2 years. It may be seen in this table that the survival of each of the 15 species of seedlings is consistently better in the sand than in the soil cultures grown under the same conditions. This increase in numbers of seedlings in the sand cultures varies from 12 per cent of the number of seedlings in the soil, in case of a less susceptible plant such as cabbage, to an increase of almost 1000 per cent in the case of spruce seedlings. The species listed in this table include both rapid-growing and slow-growing types, as is shown by the variation in the number of days (12 to 50) that the cultures were maintained. Cultures of seedlings in sand and in nontreated soil are illustrated in figure 1, A-E.

The average total numbers of seedlings that emerged in the sand and in nontreated soil cultures are shown in table 2. Here also is given the amount of post-emergence damping-off in both the sand and soil, expressed as the number of diseased seedlings per culture and also as the percentage of the total number of emerged seedlings. The number of plants emerged from the nontreated soil was generally much smaller than the number from similar sand cultures, because of preemergence damping-off in the soil.

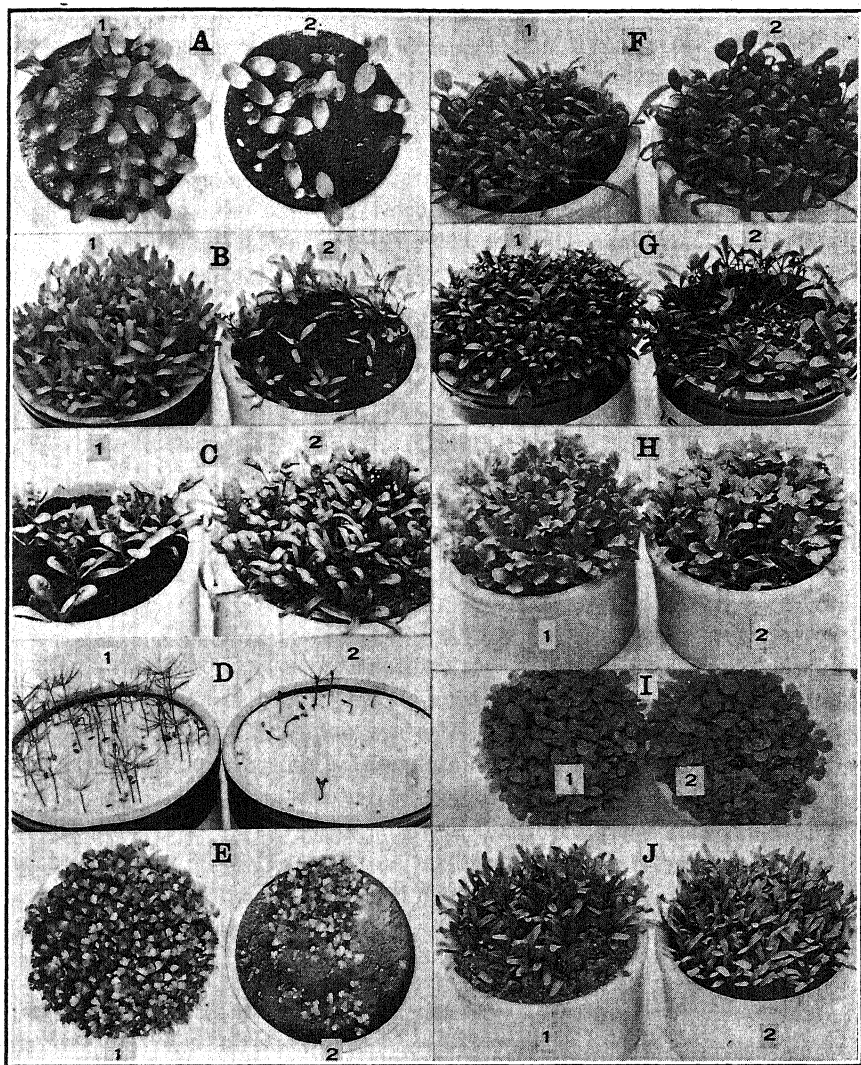


FIG. 1. Cultures of seedlings in sand and soil. A. Cucumbers: 1, sand; 2, soil. B. Beets: 1, sand; 2, soil. C. Spinach: 1, soil; 2, sand. D. Pine: 1, sand; 2, soil. E. Celery: 1, sand; 2, soil. F. Spinach: 1, soil with cuprous oxide-dusted seed; 2, sand. G. Gypsophila: 1, sand; 2, soil with formaldehyde dust. H. Lettuce: 1, sand; 2, soil with formaldehyde dust. I. Tobacco: 1, sand; 2, autoclaved soil. J. Beets: 1, sand; 2, autoclaved soil.

TABLE 2.—*Survival of seedlings after emergence, in sand and in nontreated soil per average culture*

Species	Total number of seedlings emerged		Seedlings damped-off after emergence	
	Soil	Sand	Soil	Sand
			<i>Per cent</i>	<i>Per cent</i>
Aquilegia	65	85	26	0
Beet	177	291	63	3
Cabbage	186	205	5	1
Celery	193	458	64	0
Cosmos	52	105	8	0
Delphinium	50	67	46	3
Gypsophila	241	411	64	2
Lettuce	115	267	56	0
Pepper	97	110	16	0
Pine	24	35	63	3
Salvia	67	95	17	0
Spinach	102	132	58	4
Spruce	22	107	55	2
Tomato	104	118	8	0
Zinnia	53	85	23	0

In the case of soil cultures, with autoclaved soil or with treated seeds, the amount of preemergence damping-off was found to be greatly reduced. In these cultures, however, post-emergence damping-off frequently became so very prevalent that the final number of diseased seedlings was larger than the number of such seedlings in nontreated soil.

The results of comparisons of seedling survival in sand with those of soil cultures receiving heat sterilization, formaldehyde dust, and seed treatment with cuprous oxide are given in table 3. Cuprous oxide generally was found to be more effective than the other seed treatments used in this work. As compared with the results in table 1, the amount of damping-off

TABLE 3.—*Survival of seedlings in sand as compared with seed and soil treatments*

Species	Number of healthy seedlings per culture			
	Cuprous oxide on seed	Formaldehyde dust in soil	Autoclaved soil	Sand
Beet	174	155	260	282
Cabbage	184	195	217	203
Celery	388	397	421	458
Gypsophila	130	203	312	404
Spinach	104	79	135	127
Spruce	27	31	38	105

in the soil cultures was greatly reduced by the above treatments. With but two exceptions, however, the number of healthy seedlings is larger for the sand cultures than for the treated soil or seed. These two exceptions occur in the case of autoclaved soil and represent increases over the number of seedlings in the sand cultures of only 6 and 7 per cent respectively with cabbage and spinach. The results of the soil and seed treatments, together with comparable sand cultures, are illustrated in figure 1, F-J.

In these experiments, the most consistent damping-off control has been obtained by the sand-culture method. In each of the above treatments with soil, localized areas of damping-off infection have appeared in many individual cultures. In a limited number of cases, the cultures showing such infection were set aside and the damping-off in the soil cultures was found to spread until nearly all of the seedlings were destroyed, (Fig. 1, G2). In the sand cultures, loss through fungous attack has been found to be confined to individual seedlings or to a few seedlings growing closely together.

In general the individual seedlings grown in sand have compared favorably in regard to size, root development, and vitality with seedlings from soil cultures, although they may occasionally be somewhat lighter in color. A certain amount of variation has been noted among different species as to the relative rates of growth of the seedlings in sand and in soil. In certain cases the appearance of the sand cultures at the time of emergence of the seedlings has been inferior to that of the soil. After a short period of growth, however, the sand-culture plants may equal or even surpass those grown in soil in regard to size and fresh weight. Frequently the sand cultures have been found to produce the largest seedlings.

The percentage survival of seedlings transplanted from sand into soil has been found equal to that of comparable seedlings from soil. There have been no differences noted in subsequent growth and yield between plants produced by each of these methods. Transplanting from sand is more readily accomplished and the process may be made easier by flooding the sand with water. In this investigation certain sand cultures of seedlings have been maintained for extended periods before transplanting without the plants becoming too large or overcrowded.

An important advantage of the sand-culture method is the lack of care necessary in watering the seedlings. The sand may be given a large amount of water without danger of inducing damping-off. Consequently, less frequent watering may be required than would be necessary for soil culture. Another desirable feature is that the sand may be used indefinitely, with washing in hot water before replanting for the best seedling growth and damping-off control.

The following plants have been successfully grown through the seedling stage in these experiments, in addition to those listed in the preceding tables:

Alfalfa, bean, bent grass, brocolli, carrot, cauliflower, corn, cucumber, eggplant, endive, fennel, marigold, muskmelon, parsley, parsnip, pea, petunia, snapdragon, squash, tobacco, turnips, and watermelon.

SUMMARY

Many different kinds of seedlings have been found to grow readily and develop normally in pure sand with nutrients added in solution.

Both preemergence and post-emergence damping-off have been much less in sand culture than in nontreated soil under the same conditions.

Seedling survival in sand culture has been found equal to that obtained in soil with the usual seed and soil treatments.

Certain cultural features, such as freedom from care in watering, ease of transplanting, and continued use of the substrate, are points in favor of sand culture for seedlings.

Seedlings grown in sand have been found to compare favorably with those grown in soil in regard to: rate of development, size, vigor, ability to withstand transplanting, and resistance to disease.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

TOBACCO STREAK, A VIRUS DISEASE¹

JAMES JOHNSON

(Accepted for publication April 1, 1935)

During the past 15 years the writer has repeatedly observed a disease of tobacco in the field that is characterized by a necrosis of, or along, the veins of the leaf. This disease was for many years believed to be one of the many nonparasitic leaf necroses to which tobacco apparently is subject. Usually the disease occurs on only widely scattered plants; only in one year is it recalled that it affected a sufficiently high percentage of plants to cause any concern among growers. Consequently, the disease has received no technical attention and does not appear even to have acquired a common name. The term "tobacco streak" is, therefore, proposed as perhaps the simplest and most suggestive for this malady. This disease has now been traced to a specific virus, herein described in what is believed to be sufficient detail to enable subsequent determination and differentiation from related viruses.

THE DISEASE

While tobacco streak is not a common disease, isolated specimens usually may be found in most fields examined for its presence in Wisconsin. In its most severe form it may be found on as high as 100 per cent of plants in small portions of field borders, more particularly on areas bordering the farm-yard or weedy plots. No case has been observed where the disease occurred in large percentages throughout the field.

The disease under field conditions usually is confined to the leaves, but not all the leaves on affected plants show symptoms. The middle leaves are most commonly attacked and, as a result, may be considerably dwarfed and ragged (Fig. 1). The normal tendency is for the plant to recover from the attack to such an extent that the upper leaves on an affected plant may show no symptoms whatever.

The type of necrotic lesions produced is by no means uniform in character; spots, lines, and circles with or without uniform patterns occur, more often on the basal half of the leaf than on the apical half (Fig. 2). The leaves are rarely if ever entirely killed or dropped in field plants. In the greenhouse the leaves on young inoculated plants may be nearly destroyed under conditions most favorable for the disease, but, nevertheless, recover turgidity. Vein clearing, followed by "water soaked" necrotic systemic symptoms (Fig. 3) may appear on new leaves 3 days after inoculation,

¹ Cooperative experiments of the Wisconsin Agricultural Experiment Station* and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

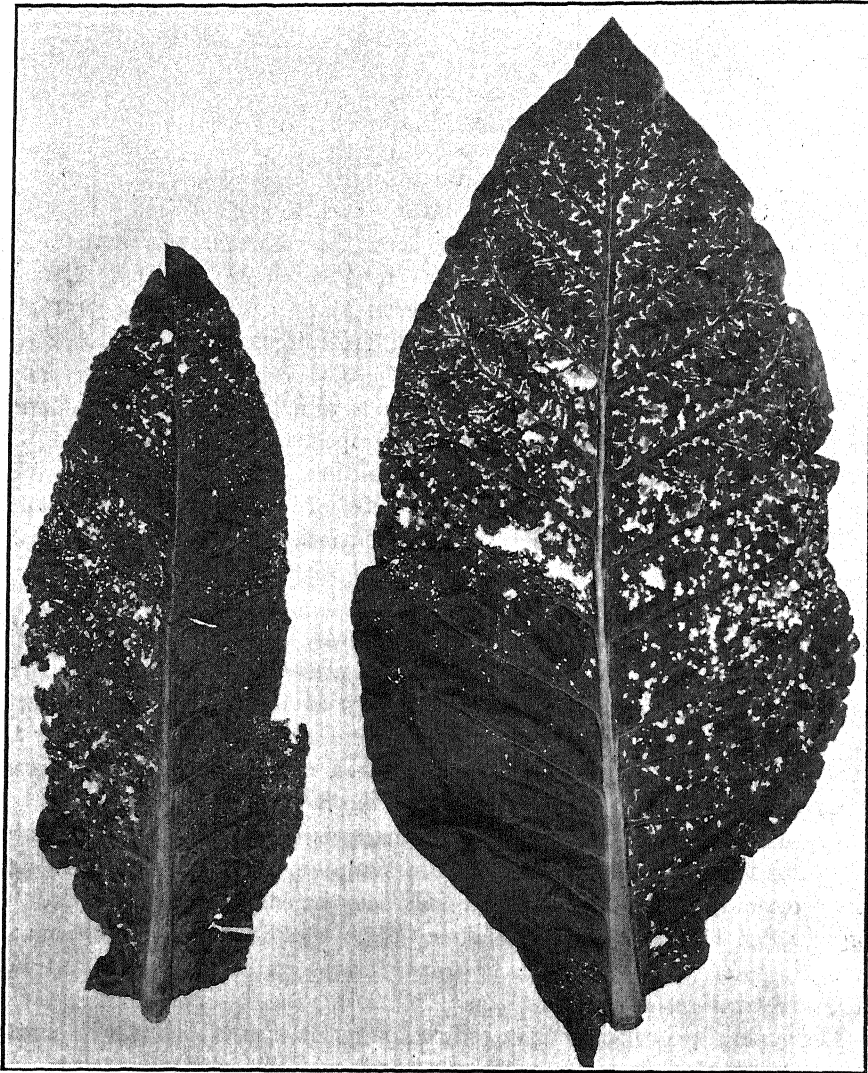


FIG. 1. Tobacco streak disease from natural field infection. The leaf to the left is greatly dwarfed as a result of early infection.

often preceded by local necrosis at the point of inoculation. Usually no mottling occurs either before or after the necrotic symptoms on tobacco. On young greenhouse plants, as in the field, the tendency for new leaves to recover is very striking, a condition that, though common with many virus diseases, is more marked in this than in any other disease observed. Recovery does not appear to bear any relation to the external environment of

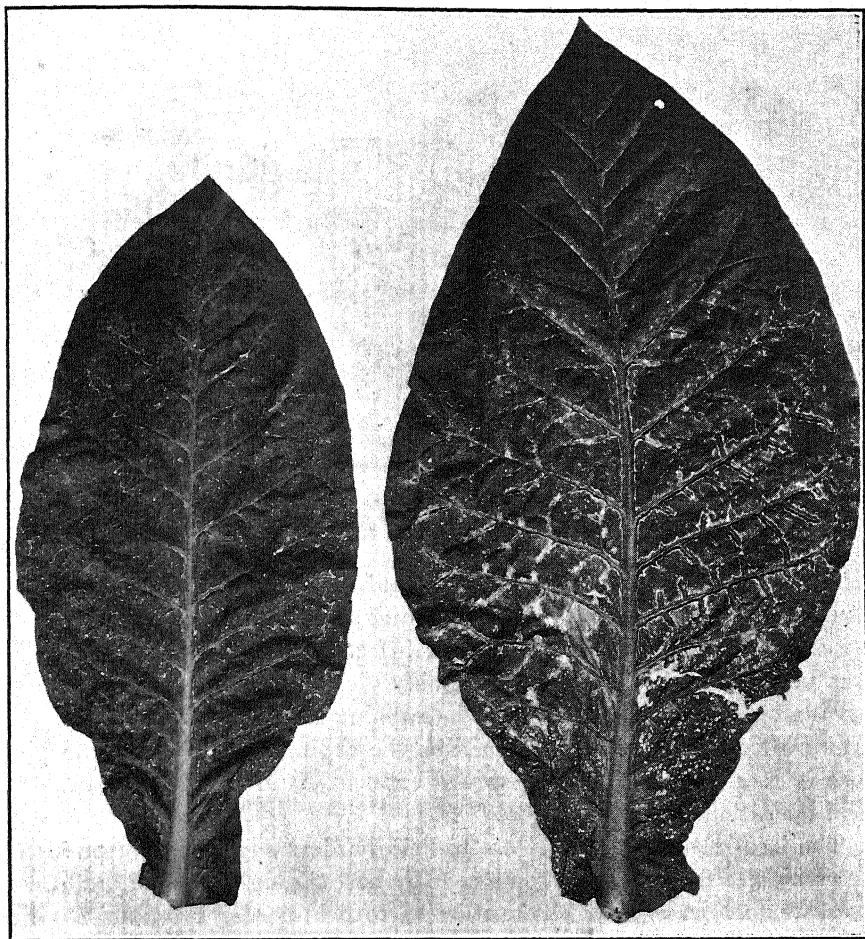


FIG. 2. Tobacco streak. The relation of infection to both the coarser and the finer veins, and the progress of the symptoms toward the apex of the leaves is shown.

the plant. Reinoculation of recovered plants, in contrast to inoculated controls, results only in vein clearing and mild mottling of the recovered leaves, and the typical necrotic symptoms fail to appear. This is apparently a case of acquired resistance to the disease, or at least to the more severe form of disease. Investigations are being continued on this phase of the problem.

THE VIRUS

Transmission.—The virus was collected from five different fields in September, 1934, and transmitted to young tobacco plants in the greenhouse without much difficulty. Previous attempts at transmission had generally

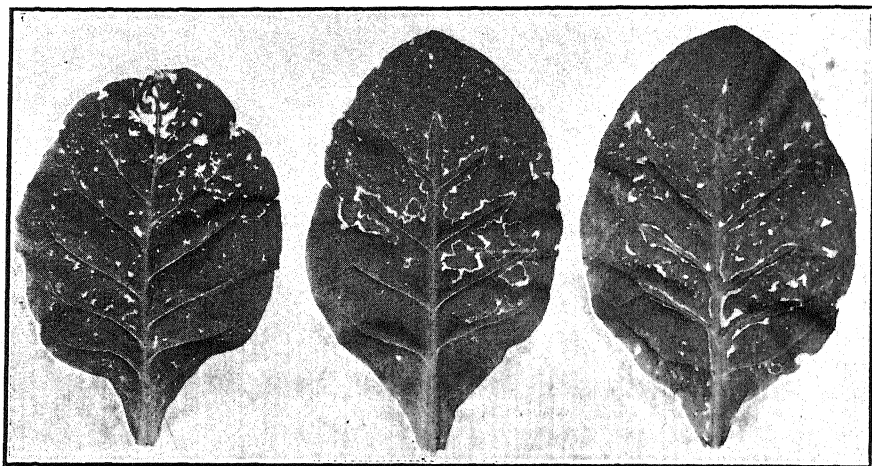


FIG. 3. Fairly typical symptoms of tobacco streak as secured on the leaves of young plants following artificial inoculation.

failed, probably because inoculum from old leaves was used. Extract from young leaves of field plants or from young suckers showing early stages of the disease gave a fair percentage of infection, and subsequent transfers from young greenhouse plants invariably yielded 100 per cent infection on Havana-seed tobacco by means of the rubbing method of inoculation. The virus evidently is not so infectious as the ordinary tobacco mosaic virus, since it does not appear to be spread extensively in the field through cultural operations.

The localization of the disease to field borders suggests transmission of the virus into the field from some near-by host through the medium of some insect vector. A similar observation as to field-border relations has been made by Thung (9) for the "curl" disease of tobacco in Java, which virus is transmitted by a species of white fly.

Although opportunity has not yet been afforded to examine such infested areas for insects, a limited number of tests have been made with certain probable vectors that were obtainable for the trials. From these tests it may be stated with considerable certainty that neither our more common aphids nor the greenhouse white fly are involved, and more limited attempts with thrips and leafhoppers have been equally unsuccessful in transmitting the virus. The following insects were used in these trials: *Myzus persicae* Sulz., *Myzus pseudosolani* Theob., *Macrosiphum solanifolii* Ashm., *Aleyrodes vaporariorum* Westw., *Thrips tabaci* Lind., a greenhouse thrips (undetermined species), and *Empoasca fabae* Harris.

Field observations do not suggest any possibility of transmission through seed.

Host Range.—No attempts have been made to transmit tobacco streak virus to plants outside the family Solanaceae. The following Solanaceae have been infected in greenhouse trials: *Nicotiana tabacum* L., *N. rustica* L., *N. glutinosa* L., *N. tabacum* × *N. glutinosa* hybrid, *Datura stramonium* L., *Nicandra physalodes* (L.) Pers., and *Physalis pubescens* L. Tomato (*Lycopersicon esculentum* Mill.) evidently is immune from infection, as is potato, *Solanum tuberosum* L.; egg plant, *S. melongena* L.; and pepper, *Capsicum annuum* L. Distinct mottling and sometimes necrosis occur on *N. glutinosa*, and mild mottling may occur on *Datura stramonium*. Since these latter hosts are attacked only locally by the ordinary tobacco-mosaic virus, whereas the streak virus may become wholly or partially systemic, they made possible a reisolation of the streak virus after this had become accidentally mixed with the tobacco mosaic virus.

Properties.—The tobacco-streak virus is relatively short-lived in extract. In 3 trials it usually was inactivated after aging for 24 hours in extract at approximately 22° C. In one trial it survived 24 but not 36 hours. Its longevity *in vitro*, therefore, may be placed at between 24 and 36 hours (Table 1). The thermal deathpoint was found to be 53° C. for 10 minutes (Table 1), and the tolerance to dilution, while not accurately determined, was low, the virus barely surviving a dilution of 1 in 20 and, probably, not extending beyond 1 in 30 (Table 1). Taking all properties into consideration, it is clear that the streak virus is very sensitive to external conditions, and, in the absence of vegetative propagation of its host, a very limited survival of the virus may be expected from year to year. On the basis of the studies conducted, the following technical description is offered for the virus causing the tobacco streak disease.

Tobacco Streak Virus.—Not transmitted by *Myzus persicae*, *Myzus pseudosolani* nor *Macrosiphum solanifolii*. Transmissible mechanically by plant extract. Resistance to aging *in vitro* between 24 and 36 hours at 22° C. Thermal deathpoint 53° C. at a 10-minute exposure. Tobacco, *Nicotiana tabacum* L., susceptible, developing local and systemic necrotic lesions on leaves, the latter symptoms being closely associated with the veins. Recovery of upper leaves is a striking characteristic of the disease of tobacco. Mottling and sometimes necrosis on *N. glutinosa* L. No infection on tomato, *Lycopersicon esculentum* Mill.

DISCUSSION

On the basis of brief symptom descriptions and illustrations to be found in the earlier literature on tobacco diseases, it is not unlikely that the disease here described as "tobacco streak" has been previously observed elsewhere as a disease of unknown cause. More recently, several virus diseases of tobacco of the necrotic type have been described, but the descriptions in

TABLE 1.—*Determinations on certain properties of the tobacco streak virus*

Longevity <i>in vitro</i> , 22° C.				Thermal death point (10 min.)				Tolerance to dilution			
Hours aged	Number of trials	Number of plants inoc.	Number of plants inf.	Temp. ° C.	Number of trials	Number of plants inoc.	Number of plants inf.	Dilution	Number of trials	Number of plants inoc.	Number of plants inf.
0	4	20	20	0	7	35	34	0	6	30	30
6	3	15	13	50	4	20	17	1-5	2	10	7
12	4	20	15	52	2	10	5	1-10	6	30	10
24	4	20	5	53	3	15	0	1-20	2	10	2
36	3	15	0	54	2	10	0	1-30	2	10	0
48	3	15	0	55	4	20	0	1-100	4	20	0

some instances have remained too incomplete as to symptoms or too limited as to the virus itself to permit a reliable comparison. While it can be definitely stated that "tobacco streak" is not to be confused with such necrotic virus diseases as tobacco ring spot (2), spotted wilt of tobacco in Australia (7), or the "Kromnek" disease in South Africa (6), it is not so definitely distinguishable at present from the Rotterdam B disease of Sumatra (3), the "vein-streak" of Sumatra described by Jochems (4), the "curl" disease of Storey (8), the "stripe and curl" of Böning (1), the "kroepoek" disease of Thung (9), or the "tobacco etch" diseases of Kentucky (5). Of the above named, the disease most closely resembles "vein-streak" of Sumatra, and, with this similarity in mind, the term "tobacco streak" has been retained for the American disease here described. As the methods and technique for virus descriptions improve, it is expected that more satisfactory comparisons of these diseases may be more readily made.

Fortunately, tobacco streak is not a sufficiently serious disease in tobacco fields to warrant any great concern about the best means of control. The discovery of the nature of the disease, however, should aid greatly in making reliable recommendations in this direction. It appears fairly clear that this virus cannot overwinter in the soil, and the localization of the disease in the fields seems to preclude any likelihood of transmission through the seed. Everything considered, it seems most likely that the virus is harbored by perennial weed hosts in the neighborhood of the tobacco fields. Considering the frequency of the occurrence of the disease, even though in very limited amounts, the alternate host must be a fairly common one, and it should not prove difficult to locate such an overwintering host if it actually exists. The transmitting agent from such a possible host or hosts also remains unknown. It is clear that experiments on the control of the disease might well be directed toward the discovery of a possible overwintering host and a natural transmitting agent.

SUMMARY

Tobacco "streak," a relatively rare disease of tobacco, is shown to be caused by a virus. The disease is characterized by striking necrotic leaf patterns, usually bearing a close relation to the veins. The disease is not new, but the nature of the malady has remained obscure in the past.

The virus is mechanically transmissible by plant extract, but no insect vector has been found. The properties of the virus show that it is relatively sensitive. It withstands aging *in vitro* less than 36 hours, the thermal death point is 53° C., and the tolerance to dilution is less than 1 in 30. Several other solanaceous species are susceptible to the virus, while others, like the tomato, apparently are immune.

The control of the disease probably will remain dependent upon the discovery of a natural overwintering host and a possible insect vector.

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PHYTOPATHOLOGICAL NOTES

A Method for Staining Rust Mycelium in Woody Tissues.—During the course of a study of Scotch pine, infected with a species of *Peridermium*, the author² had occasion to use with good success the orseillin BB-anilin-blue-staining procedure described by Strasburger, pp. 392 and 767.⁴

The staining of rust mycelium and haustoria in tissues was so effective that the method suggested itself as valuable in the routine microscopic diagnosis of the white-pine blister rust. The method perhaps most often used for this purpose at present is the short method described by Colley¹ with the use of safranin and light green. The Strasburger procedure has been adapted to the shorter Colley method, so that sections may be stained in a comparatively short time.

Sections of white pine bark and wood, cut on a freezing microtome or by hand, are placed in water without previous fixation. The water is replaced by a saturated solution of orseillin BB³ in 3 per cent acetic acid that is allowed to remain for 10 to 12 hours. The sections are then rinsed in 40 per cent ethyl alcohol in order to remove excess stain. A saturated solution of anilin blue in 3 per cent acetic acid is then added and allowed to remain for 15 to 30 minutes. A rapid rinsing in 40 per cent ethyl alcohol to remove any excess stain is followed by the addition of 95 per cent alcohol for 2 to 5 minutes. This is followed by absolute alcohol, which in turn, is replaced by fresh absolute alcohol. The sections may then be transferred to clove oil in which they may be examined if the mount is to be temporary, only. The clove oil may be washed away by xylol, and balsam or gum damar added if the sections are to be mounted permanently.

The most satisfactory results are usually obtained when differentiation with 40 and 95 per cent of ethyl alcohol has been carried on, so that the sections appear a rather light purple. It will be found necessary to alter the time schedule somewhat according to the thickness of the sections and according to the amount of resin the pine contains, as portions abundant in resin stain heavily with orseillin.

In properly differentiated tissues the mycelium stains violet to blue, lignified or suberized tissue stains red, parenchyma cell walls usually blue,

¹ Colley, R. H. Diagnosing white-pine blister-rust from its mycelium. Jour. Agr. Research [U. S.] 11: 281-286. 1917.

² Hutchinson, W. G. Resistance of *Pinus sylvestris* L. to a gall-forming *Peridermium*. Phytopath. 25: 819-843. 1935.

³ Orseillin BB may be obtained from Akatos Inc., 55 Van Dam St., New York, N. Y. It is not the same stain as orsein.

⁴ Strasburger, E. Das botanische Praktikum. 7th Ed. 883 pp. G. Fischer, Jena. 1923.

and nuclei and cytoplasm red. Haustoria, penetrating tracheids and surrounded by lignified callosities, stain deep red in contrast to the bluish mycelium outside the cell wall.

Comparison of blister-rust-infected tissues stained by the above method with similar tissues stained with safranin and light green shows that in the former case the mycelium is much more obvious and clear-cut. Even in dried woody tissues, it is a simple matter to recognize the rust mycelium.—W. G. HUTCHINSON, Department of Botany, University of Pennsylvania, Philadelphia, Pa.

The Status of the Nematode Aphelenchus avenae Bastian, 1865, as a Plant Parasite.—There are considerable numbers of nematode species other than obligate plant-parasitic forms, which are found associated with diseased plants more or less frequently,—some even regularly. Their status as disease agents is in most instances doubtful. Most of these forms are thought to be connected only with decay, a viewpoint which is supported by the fact that they can exist in or on decayed material and do not need healthy tissue, as do the obligate plant parasites. However, observations and a few experiments seem to prove that this view does not entirely cover the actual situation. The connection of these forms with various diseased conditions in plants is of a more serious nature than is generally conceived. It may be that many of these forms live as saprophytes, but they undoubtedly are able to attack and to damage healthy plant tissues and to propagate in tissue not yet decayed. Contrary to the views generally expressed in this regard¹ concerning the significance of *Aphelenchus avenae*, our observations in numerous instances have proved again and again that *A. avenae* is not a harmless form. In diseased plant material submitted to the Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture, *Aphelenchus avenae* is one of the most frequently observed species, often associated with other nemas but often also found as the only form present. It may perhaps live on necrotic plant tissue, but it undoubtedly can live on fungi and on healthy plant tissue. This form is to be considered as a facultative plant parasite with perhaps dominant necrobiotic food tendencies. To substantiate this view a sagittal section through the root of a phlox hybrid² is illustrated, showing in the parenchyma of this root tissue 2 specimens, a larva and an adult female of *A. avenae*, with 5 eggs scattered through the tissue, one of the eggs apparently having just been deposited (Fig. 1). *Aphelenchus avenae* undoubtedly migrates through the tissue and, of course,

¹ Goodey, T. The pathology and aetiology of plant lesions caused by parasitic nematodes. 34 p. Imp. Bur. Agr. Parasitol. St. Albans, Eng. 1935.

² Received through the courtesy of Mrs. Flavia H. Moise, of the U. S. Dept. of Agriculture. The plant is a hybrid of *Phlox glaberrima suffruticosa*.

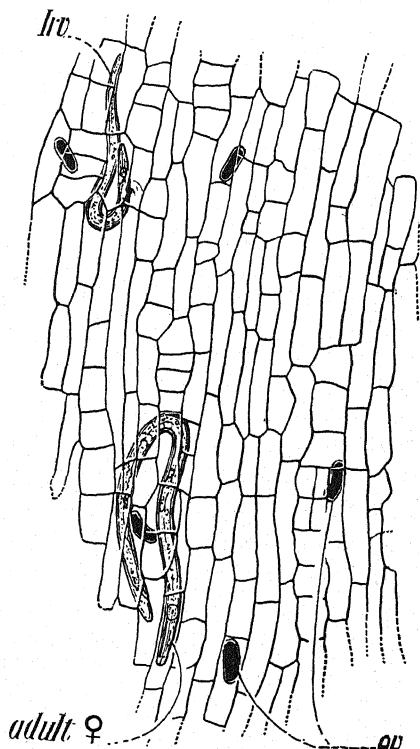


FIG. 1. *Aphelenchus avenae* female, larva and eggs in situ in parenchyma of cortex of phlox root; lrv larva; ov eggs. $\times 80$.

in this way must inflict mechanical injury. It is thought that it feeds on the cell contents of the host tissue, but it is unknown whether only a certain part of the cell content is sucked in or the whole. In addition, the waste products of its metabolism are discharged into this plant tissue and no doubt interfere with its normal functions. The fact that the female specimen figured had deposited at least 5 eggs throughout the tissue undoubtedly proves that the nematode is able to sustain life there and is in this instance a parasite.—G. STEINER, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

BOOK REVIEWS

Curtis, Otis Freeman.—*The Translocation of Solutes in Plants: A Critical Consideration of Evidence Bearing upon Solute Movement.* xiii + 273 pp. Illus. diagrs. McGraw-Hill Book Co., Inc.: New York and London. 1935. \$3.00.

Professor Curtis, after 17 years of work on problems associated with translocation of solutes in plants, has at last assembled his material in book form. It will be recalled that, while orthodox phytophysiological dogma includes among its tenets the doctrine that mineral salts and other soluble materials (including sugars) travel upward from the roots to the growing organs through the xylem and that the manufactured foods (carbohydrates and proteins) travel downward from the leaves to the roots via the phloem, there are some heterodox iconoclasts among plant physiologists who do not subscribe to this particular article of faith; included in this small group of unbelievers are H. H. Dixon and O. F. Curtis.

The former holds that the xylem is the chief channel for both upward and downward movement, and relegates the phloem to a rather unessential role as far as translocation is concerned. Curtis, on the other hand, while admitting that the xylem does have a function in carrying water from the roots to the leaves, believes that the phloem carries nitrogen compounds and salts upward as well as other soluble nutrients and foods, in addition to its commonly accepted function of downward transportation. The seven chapters of this book set forth the author's reasons for this belief, with an explanation of how this double-dealing is accomplished and a short but pertinent discussion of some cognate problems.

After a brief introduction (Chap. I), the author launches into a presentation of the evidence for the upward transport, through the phloem, of organic matter (Chap. II) and of nitrogen and salts (Chap. III). These two are by far the most important and interesting chapters in the book. They contain the essence of the Curtisian creed, and include a comprehensive description of the author's ringing experiments, which certainly indicate that, in the plants described and under the conditions given, the phloem carries the burden of the substances under discussion. There seems to be no other alternative. The problem is too complex to consider in detail here, although one cannot help but wish that more space were given to an explanation (or refutation) of the classic experiments upon which the orthodox beliefs are based. It is easier for one to believe Curtis' explanation of his particular experiments than it is to throw overboard the more conventional ideas; in other words, it still remains to be shown that his conclusions are of universal application and can be generalized for all plants and under normal conditions.

There follows a confutation (Chap. IV) of the Dixonian doctrine, mentioned above; and here Curtis will have with him most of his readers. At this point is then presented (Chap. V) the criteria for determining tissues of transport, wherein it is pointed out that the more commonly accepted criteria for determining the region of translocation, such as the movement of solutes introduced through cut tissues or the presence of a substance in a tissue, are not valid in that they do not *prove* that these tissues conduct the materials in question. This is all too true, but physiological science still contains so many unknowns that "sweet reason" yet remains a virtue; and it is not hard to see that Curtis is a little more severe in demanding *absolute* proofs when treating the theories he is seeking to displace than when dealing with his own hypotheses.

But by this time the reader is supposed to be convinced that the phloem is the logical (and experimentally proved) channel of both upward and downward translocation. The question as to *how* this is accomplished is answered in the following sections (Chap. VI). In this excellent discussion of the problems involved, including a critique of the contributions and theories of Münch and Crafts, it is suggested that the chief factor in translocation is some sort of streaming and rotational movement of the protoplasm in the phloem (sieve tubes), which occurs in such a way that descending materials are fed in at the tops of the cells and ascending materials in at the bottom. While such a system seems possible, the difficulties are so great that one can only conclude that if plants don't use the transpiration stream in the xylem for their ascending materials they are missing a big chance to save themselves a lot of trouble!

While readers, in general, will approve of the author's discussion and conclusions, it seems to the present reviewer that Curtis has not always distinguished carefully (*e.g.*, in discussing Münch's hypothesis) between mass diffusion and osmotic gradients. The former deal primarily with proteins and other materials of such molecular size (colloids) that they do not exert osmotic pressure. *Osmotic* gradients, therefore, do not enter into the question of the diffusion of proteins, and the objection that such materials in passing downwards from the leaf go "against the osmotic gradient" does not hold. Also one must not forget that each substance exerts its own osmotic pressure independently of the others present; it is hence quite possible for the total osmotic pressure in "receiving cells" to be higher than that in "supplying cells" (see p. 152) and yet for the concentration of some individual component, *e.g.*, KNO_3 , to be lower in the former. Furthermore, one must question the statement on page 151 that "Although in trees there seems to be a tendency for an increase in osmotic concentration of leaves at greater heights, this increase is not uniform, is at best not very great, and seems to bear no relation to the distance through which foods must move."

Dixon and Atkins (1916) found at the end of February an increase of osmotic pressure in the wood of *Acer macrophyllum* of about 0.2 atmosphere for each meter of elevation above the ground; and Harris and his coworkers, as well as others, have found considerable (and regular) increases in the osmotic pressure of leaves as one goes from the base of the crown to the top.

In a final chapter (VII) is discussed the relation of the translocation of solutes to such kindred questions as the movements of hormones, polarity, and regeneration, these phenomena being interpreted in the light of the hypothesis of Curtis. An extensive (indexed) bibliography and subject index conclude the volume.

From the point of view of the plant pathologist, the principal subject discussed is the movement and path of viruses. Evidence reviewed seems to show that the viruses commonly are carried in the phloem. Results of some workers indicate an association of the virus movement with that of carbohydrates and that the movement is unidirectional; but still other results show the movement to be independent of the movement of nutrients and to be in both directions at the same time. There would, hence, seem to be no uniformity in this matter; each virus has a specific behavior.

The book is splendidly written and printed, with few typographical errors, *e.g.*, an incorrect spelling of *pseudoacacia* on p. 161 (spelled correctly, however, on p. 180). The style is clear-cut; on the whole, logical; concise; and eminently readable. The summaries of the various sections at the end of each chapter are decided aids to the reader in extracting the essence of the text, and are models of condensation and of the abstractor's art. Few writers seem able to summarize their own work so admirably. But why do the publishers print "First Edition" on the title-page? Isn't it understood that, unless otherwise stated, the edition is the first? Or is this intended as an implication that there are more to follow?—ORAN RABER, Plant Physiologist, Forest Service, U. S. D. A.

Wardlaw, Claude Wilson. *Diseases of the Banana and of the Manila Hemp Plant*. 615 pp. 2 col. pl. illus. Macmillan & Co., London, 1935. \$8.00. (30 s.).

The author of this work indicates in his preface that precise knowledge of the maladies affecting bananas is far from complete and that the task of selecting it from the voluminous but scattered mycological and agricultural literature on bananas has been one of no little magnitude. He has accomplished the task with rare discrimination and has presented an admirable summary of reliable contributions on this important crop. The book is carefully edited and abundantly illustrated with photographs and line drawings, including many from his own original papers. About a fourth of the large volume is devoted to banana wilt or Panama disease caused by *Fusarium*

oxysporum cubense, the most widely known and injurious disease of bananas in the American tropics. In addition, plantation diseases of the fruit and leaf, and storage diseases of the fruit are exhaustively treated. Somewhat briefer reviews of the diseases of abacá and deterioration of the fiber are presented. The most recent as well as the earlier literature has been assembled and sifted in the light of up-to-date research; and the information presented represents current thought of investigators in the field of banana husbandry and transportation. An interesting feature of the section on banana wilt is a short account of attempts to breed for resistance, which is complicated, because the banana is a sterile plant propagated by vegetative offsets. Reproductive organs of both sexes are abortive to an extent of more than ninety-nine per cent. Success of the program of improvement by breeding depends upon the fact that seeds may develop under special conditions, but it is essential of course that the new varieties intended for commercial use do not have seeds. There is reason for believing that, when grown in pure stands, even the small percentage of normal sexual cells formed have no chance of functioning, because of self-sterility. Resort was had in the earlier work to crossing with the highly fertile pollen of seed-bearing species of *Musa*. Hybrid seedlings, apparently immune from wilt, were produced in this way, but the disadvantage is the presence of an occasional seed. Cytological observations make it reasonable to conclude that triploid seedlings of commercial usefulness may be produced by synthesis from diploids or by reduction from tetraploids.

The author concludes the book with four appendices containing lists of bacteria and fungi associated with the banana plant as saprophytes and parasites, cultural characteristics of strains of *Fusarium cubense*, statistics on imports of bananas into Great Britain, and data on environmental conditions in the holds of banana boats. Without question, he deserves credit for one of the best presentations of disease hazards and control measures to be found in plant pathological literature relating to tropical crops.—E. W. BRANDES, Bureau of Plant Industry, Department of Agriculture, Washington, D. C.

Tehon, Leo Roy. *A Monographic Rearrangement of Lophodermium*. 151 pp. Illinois Biological Monographs, Vol. XIII, no. 4. (Univ. of Illinois, Bul. v, 32, no. 51, Aug. 20, 1935.) 1 illus. 5 plates. 1935. Price \$2.00.

The bringing together in one accessible publication of a large body of data scattered through the mycological literature of many countries and covering a century or more is an undertaking to be commended, even though one is not able to agree with all phases of the treatment as presented by the author. The publication under review covers a detailed study of the mate-

rial available to the author of the genus *Lophodermium* here assigned to the family Hypodermataceae. The author points out that the genus is one that has not been thoroughly investigated as to its taxonomic relationships, and he gives a summary of studies made to date by other mycologists, with special reference to the work of Fries, Rehm, von Hoehnel, and Darker. A very detailed account of the morphology of the hysterothecium and other structures involved, based to a considerable extent on the author's own studies, follows.

In the systematic section constituting the larger portion of the paper 4 new species and one new combination are recognized in the genus *Lophodermium* proper, with one species renamed. As segregates from *Lophodermium* the author names as new the genus *Dermascia* and takes up two names proposed, but not described by von Hoehnel, namely, *Lophodermellina* and *Lophodermina*. These segregations are based in large part on the controversial von Hoehnel system, which uses the general position of the ascocarp with relation to the host tissue, *i.e.*, whether subepidermal or intraepidermal, as a basis of generic distinction. In *Dermascia*, one new species and 13 new combinations are made, in *Lophodermellina* one new species and 5 new combinations, in *Lophodermina* 2 new species and 20 new combinations. Descriptions, both of the several genera discussed and the species themselves, appear to be complete and carefully drawn and few typographical errors have been noted. A host list enhances the value of the publication to phytopathologists.—J. A. STEVENSON.

REPORT OF THE OFFICIAL REPRESENTATIVE COMMITTEE
OF THE SOCIETY, SIXTH INTERNATIONAL BOTANICAL
CONGRESS AND INTERNATIONAL UNION OF
BIOLOGICAL SCIENCES, SEPTEMBER
1935, AMSTERDAM

International Union of Biological Sciences. Adherence of the United States to this International Union was made possible by action of the National Research Council during the summer of 1935. It fell to members of your committee to cast the votes of the United States at the Amsterdam meeting on September 1, due to the fact that no other American societies had arranged for accredited representatives.

An American botanist, Doctor E. D. Merrill, of Harvard University, (recently of the New York Botanical Garden) was elected president of the Union.

A subsection of phytopathology was authorized in the Botanical Section. Doctor Donald Reddick, Cornell University, was elected Vice-President of this subsection.

Several other subsections were authorized including a Subsection of Applied Botany. The latter was authorized with the distinct understanding that it should not include phytopathology. Your representatives voted against the organization of this subsection and also against the organization of a Subsection on Forestry which was defeated.

Sixth International Botanical Congress. Members of your committee attended all sessions of the Subsection on Nomenclature, including the special sessions held by the mycologists, as well as all sessions of the section on phytopathology. The results of all official actions taken at the various meetings will be published elsewhere in detail.

The actions of chief interest to this Society were:

(1) *General Resolution.*

It is proposed that the Botanical Section of the International Union of Biological Sciences should act as an administrative connecting link between the successive International Botanical Congresses, which maintain their full independence from an international point of view to such an extent that any country which has not joined the Union, will have quite the same rights as those which have joined.

It is proposed that this Botanical Section be authorized to carry through any resolutions, carried by the International Botanical Congresses.

Proposed by A. C. SEWARD and seconded by J. C. SCHONTE, E. J. BUTLER, E. D. MERRILL, N. NÉMEC, M. J. SIRKS and N. E. SVEDELIUS.

(12) *Sections Myc. and Path.*

The Sixth International Botanical Congress wishes to express its sincere appreciation of the admirable work accomplished by the Centraalbureau voor Schimmelcultures at Baarn since its foundation in 1906 and views with grave concern the present financial difficulties of what is essentially an international institution.

Proposed to the Section MYC by J. RAMSBOTTOM and seconded by E. J. BUTLER; to the Section Path. proposed by E. RIEHM and seconded by E. C. STAKMAN.

(13) *Section Path.*

The Committee on Description and Nomenclature of Plant Viruses appointed by the Fifth International Botanical Congress 1930 wishes to report that it has made progress in developing a scheme for the nomenclature of plant viruses and suggests to this congress that the Committee be empowered to continue its consideration and establish an acceptable system of virus nomenclature.

Proposed by H. M. QUANJER and seconded by JAMES JOHNSON, P. A. MURPHY, J. HENDERSON SMITH and G. SAMUEL.

(14) *Section Path.*

It is recommended that the term "physiologic race" be substituted for "physiologic form" as the former seems more appropriate. It is recommended further that the word "race" be used in general to designate biotypes or groups of biotypes that differ from each other in physiologic characters.

Proposed by E. C. STAKMAN and seconded by Miss M. NEWTON, Miss G. MILBRINK and TH. ROEMER.

(15) *Section Path.*

The Sixth International Botanical Congress expresses itself of the opinion:

That an effective and unceasing campaign against destructive plant diseases, insects and pests can be successfully prosecuted only by international action and mutual cooperation;

that close and frequent international discussion of the problems of plant quarantines should take place to bring about improvement of the health conditions of plants and plant products offered for export;

that it unanimously recognizes that such action will greatly facilitate international trade in the commodities concerned, and

that, finally, this resolution be brought to the attention of the League of Nations, emphatically endorsing the League's proposal to give this matter the urgent and careful consideration with a view to facilitating and expediting the purpose and aims of this resolution.

Proposed by H. T. GÜSSOW and seconded by N. VAN POETEREN.

The Seventh International Botanical Congress will be held in Sweden in 1940.

For the Committee,

H. T. GÜSSOW, *Chairman*.

PHYTOPATHOLOGY

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CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

VI. THE ISOLATION FROM DISEASED TURKISH TOBACCO PLANTS OF A CRYSTALLINE PROTEIN POSSESSING THE PROPERTIES OF TOBACCO- MOSAIC VIRUS¹

W. M. STANLEY

(Accepted for publication November 7, 1935)

During the past few years rather extensive chemical studies have been made on juices from tobacco plants infected with tobacco-mosaic virus. During the course of these investigations it was found, in experiments with pepsin (15) and with certain protein precipitants (16), that this virus acts as a protein. As a result of chemical work, Vinson and Petre (18, 19) and other workers (1, 4) believed tobacco-mosaic virus to be a non-living chemical agent, although certain investigators have regarded it and other viruses as living agents (3, 5). Rivers (11) and Burnet and Andrewes (3) have published reviews and bibliographies relative to the nature of viruses. The evidence in support of a belief in the protein nature of tobacco-mosaic virus seemed sufficiently strong to warrant a further study employing chemical methods. This study resulted in the isolation of a crystalline protein having the properties of tobacco-mosaic virus (17). The present paper presents in detail the methods of concentration and purification and the general properties of this protein.

EXPERIMENTAL

Preparation of Crude Globulin

The active crystalline protein has been isolated from several different lots of infected plants, some of which were grown in the greenhouse at different times during the summer and winter and some of which were grown outside in the field during the summer. The description and data included in the following paragraphs are for infected plants grown in a field during the past summer.

¹ Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in no wise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

Small Turkish tobacco plants about 4 to 6 inches high were planted in a field during the week of May 20, 1935. The plants were inoculated with the ordinary strain of tobacco-mosaic virus (Johnson's tobacco virus 1) on June 13, 1935, by rubbing one leaf of each plant once with a bandage gauze pad moistened with a preparation consisting of infectious juice diluted with 100 parts of water. When the plants had reached a stage of maturity indicated by the beginning of flower-bud formation, which was during the week of July 15, 1935, they were cut off about 6 to 10 inches above the ground, placed in large burlap bags, and immediately transferred to a room held at a temperature of -8° C. The stalks in the ground sprouted new shoots and about a month later a second crop of infected plants was cut and stored. A third crop was obtained after about another month. The second crop gave the largest harvest and the third crop the smallest.

As soon as the plants were completely frozen they were removed, a bag at a time, and immediately, while still frozen, put through a large power meat grinder.² It was found much easier to grind the frozen material than either the fresh material or the frozen and thawed material. The macerated pulp was caught in 5-gallon tinned dairy pails.³ To each pail of pulp was then added 750 cc. of a solution prepared by making 20 lb. of granular U. S. P. disodium phosphate up to 15 liters with hot tap water. It was found necessary to have the pail, in which this solution was prepared, immersed in hot water for a few minutes in order to get all of the disodium phosphate into solution. The pulp and the disodium phosphate solution were thoroughly mixed by hand and then allowed to stand overnight in order to allow the frozen pulp to thaw thoroughly.

The pulp was then transferred to large bags made of 4 layers of bandage gauze and holding one pail of pulp each. These bags were suspended from a hook and after the excess liquid had drained off some additional liquid was obtained by turning and pressing the bags by hand. The bags were then subjected to pressure in a fruit press⁴ of sufficient size to hold one bag, and additional liquid was pressed out. All of the liquid obtained from the pulp in this first extraction was combined and placed in 5-gallon tinned pails. The fruit press, which was found preferable to a small hydraulic press, brought the yield of juice up to about 70 per cent of the volume of the pulp. The pails were covered and placed in a cool room or in a low-temperature room held at -8° C. The press cakes of pulp from the first extraction, each representing one pail of the original macerated material, were immediately thoroughly mixed with a volume of cold tap water equal to that of the first extract. Then 750 cc. of the disodium phosphate solution was added to each

² Model No. 432SP obtained from The Enterprise Mfg. Co., Philadelphia, Pa.

³ No. 5550 obtained from The Atlantic Stamping Co., Rochester, N. Y.

⁴ Similar to No. 20 of the Baccellieri Mfg. Co., Berlin, N. J.

pail of pulp mixture. The covered pails were allowed to stand overnight in the low-temperature room. The next morning they were transferred to bags and a second extract obtained by pressing by hand and by means of the fruit press. The second extract was allowed to stand in the low-temperature room overnight and the press cakes from the second extract were discarded. The procedure just described, involving 2 extractions and the use of disodium phosphate, which was used to decrease the hydrogen-ion concentration of the extracts to between pH 6 and 7, was found to yield from 2 to 10 times more virus than any of the other methods used.

After standing overnight, the first extract was decanted from a small amount of green-colored precipitate, about 200 gm. of celite (Hyflo Super-cel⁵) was added to each pail of extract, and, after stirring, the mixture was filtered through a $\frac{1}{4}$ -inch layer of celite on a large Büchner funnel. In order to facilitate filtration, it was found advisable to remove occasionally the uppermost layer of the filter cake from the Büchner funnel. The celite thus removed was not discarded but was added to the pails of extract to be filtered. It was found possible to use the same celite several times before it slowed the filtration sufficiently to necessitate discarding it. The addition of celite to the freshly pressed juice permitted rapid filtration of the juice through the layer of celite on the Büchner funnel. This operation gave a sparkling clear brownish filtrate. Table 1 records the data concerning the total solids, ash, total nitrogen, and protein nitrogen of juice from the first harvest before and after filtration through celite and the same data for the

TABLE 1.—*Total solids, ash, total nitrogen and protein-nitrogen determinations on dilute disodium phosphate extracts from frozen, macerated, infected Turkish tobacco plants before and after filtration through celite^a*

	Mg. total solids per cc. ^b	Mg. ash per cc. ^b	Mg. total N per cc. ^b	Mg. pro- tein-N per cc. ^b	Mg. total N per cc. ^c	Mg. pro- tein-N per cc. ^c
1st extract before filtration	47.1	19.3	2.96	1.07	2.65	1.24
1st extract after filtration	43.9	17.9	2.96	0.87	2.65	1.18
2nd extract before filtration	34.6	20.8	2.08	0.70	2.20	0.47
2nd extract after filtration	32.6	19.6	1.78	0.45	2.02	0.42

^a Infectivity data are given in table 2.

^b Data from first crop of plants.

^c Data from second crop of plants.

⁵ All celite preparations were obtained from Johns-Manville, 22 East 40th Street, New York, N. Y.

second extract of the pulp. Total nitrogen and protein-nitrogen data for juice from the second harvest are included. The table shows that there was only a slight loss of protein on filtration through celite. There was also a slight reduction in total solids, total nitrogen and ash in most instances. Although the second extracts contained almost $\frac{1}{2}$ as much protein as the first extracts, it was found that a third extraction gave only about $\frac{1}{10}$ as much protein as the first extraction. Therefore, the macerated material was subjected to only 2 extractions. The infectiousness of the first and second extracts before and after filtration was determined by means of inoculations to leaves of *Nicotiana glutinosa* L., using the half-leaf method (6, 13, 14). The results, given in table 2, show that there is no great difference in the

TABLE 2.—*Infectivity tests on 2 dilute disodium phosphate extracts from frozen macerated infected Turkish tobacco plants before and after filtration through celite^a*

Dilution →	1	1-10	1-100	1-1000	1-10,000	1-100,000	1-1,000,000
1st extract before filtration	29.7 ^b	30.0	18.3	8.9	0.6	0.1	0.0
1st extract after filtration	32.4 ^c	36.0	45.0	10.2	0.5	0.0	0.0
2nd extract before filtration	52.5	24.5	21.0	5.8	2.1	0.0	0.0
2nd extract after filtration	45.7	19.8	22.4	10.0	1.9	0.1	0.0
1st extract before filtration	40.0	23.4	10.7	10.0	0.2	1.1	0.0
2nd extract before filtration	59.6	31.8	12.5	10.3	0.3	0.6	0.0
1st extract after filtration	34.7	47.1	18.2	14.6	0.7	0.1	0.0
2nd extract after filtration	61.3	52.7	16.3	7.2	0.8	0.2	0.0

^a Analytical data are given in table 1.

^b Numbers represent the average number of lesions per half-leaf obtained on the left halves of 5 leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution. Dilutions were made with distilled water.

^c Numbers represent the average number of lesions per half-leaf obtained on the right halves of the same leaves on inoculation with the designated preparation and dilution. Dilutions were made with distilled water.

infectivity of these 4 preparations. Thus the protein content and the infectivity of the extracts are about the same before and after filtration through celite.

To each pail containing about 15 liters of 1st-extraction filtrate was added about 200 cc. of 3M H_2SO_4 , with vigorous stirring, in order to adjust the hydrogen-ion concentration to about pH 5. Smaller amounts of 3M H_2SO_4 (from 100 to 140 cc.) were used to adjust the 2nd-extraction filtrate to the same hydrogen-ion concentration. The globulin fraction was precipitated by adding, with stirring, 3 kg. of ammonium sulphate to each pail (9, 18), and the mixture was then placed in a cool room overnight. The mixtures were then filtered through folded filter paper, using 10-inch glass funnels and 50 cm. C. S. and S. No. 1450 $\frac{1}{2}$ paper. Much more color was lost in the filtrate when this type of filtration was used than when the filtration was carried out on a Büchner funnel with suction, using either paper or paper and celite. About one pail could be filtered through one paper during 24 hours. During this and all subsequent operations the activity of the precipitates and of the filtrates was repeatedly checked by means of infectivity tests on leaves of *Nicotiana glutinosa*. The dark-colored filtrates were found to contain little or no virus and were discarded. The dark brown paste-like precipitates were removed from the filter paper by means of a spatula, combined, and placed on large Büchner funnels in order to remove as much of the colored fluid as possible. The papers from which most of the globulin had been removed were collected, extracted with dilute disodium phosphate solution, and the extract adjusted to about pH 5 and to about 0.4 saturation with ammonium sulphate. The precipitate was removed by filtration and added to the main portion.

After being subjected to filtration with suction on the Büchner funnel for a day, the dark brown globulin fraction was divided into lots of from 200 to 300 gm. Each lot was suspended in 15 liters of water in a pail to which 0.5 kg. disodium phosphate was added. The hydrogen-ion concentration was adjusted to about pH 8 by the addition, with stirring, of about 300 cc. of 2N NaOH per pail of mixture. To each pail was then added 200 gm. of Hyflo Standard-cel, with stirring, and the mixture was then filtered with suction through a $\frac{1}{4}$ -inch layer of the same material on a large Büchner funnel. Data on the protein content of many different preparations before and after filtration through either Hyflo Super-cel or Hyflo Standard-cel indicated that from 5 to 30 per cent, with an average of about 20 per cent, of the protein remained on the filter cake. Although it was found possible to recover practically all of this protein by 1 or 2 extractions of the celite filter cake, this was not done routinely because the amount of the recovered protein did not warrant the added labor involved in the recovery.

Following the filtration through celite, the filtrate was adjusted to pH 5 by the addition of about 150 cc. of 3M H_2SO_4 . Three kg. of ammonium sulphate were then added to each pail of filtrate. After standing in a cool room overnight, the precipitate was collected by filtration using folded C. S.

and S. No. 1450 $\frac{1}{2}$ paper. This procedure, in which the globulin fraction was taken into solution, filtered through celite and again precipitated, was repeated from 2 to 4 times in order to obtain a filtrate from the precipitated globulin that was practically colorless. The precipitate was still dark brown, despite the fact that much color had been lost in the filtrates from the precipitated and reprecipitated globulin. Table 3 records the tests for

TABLE 3.—*Infectivity tests on once-precipitated globulin from first extract, once-precipitated globulin from second extract, and on 5-times-precipitated globulin*

Dilution →		1-10 ³	1-10 ⁴	1-10 ⁵	1-10 ⁶	1-10 ⁷	1-10 ⁸	1-10 ⁹
Once-precipitated globulin. 1st extract.	<i>P. vulgaris</i>	340.5 ^a	236.0	114.0	64.5	19.2	3.9	1.1
	<i>N. glutinosa</i>	144.0 ^a	84.3	60.6	45.5	10.2	3.2	0.2
Once-precipitated globulin. 2nd extract.	<i>P. vulgaris</i>	385.0	382.5	59.6	60.0	3.2	2.2	1.3
	<i>N. glutinosa</i>	182.0	175.0	134.0	33.0	8.0	0.8	1.8
5-times-precipitated globulin from mixture of extracts.	<i>N. glutinosa</i>	275.5	132.5	133.5	26.7	15.8	8.9	2.6

^a Numbers represent the average number of lesions per leaf obtained on 10 or more leaves of *Phaseolus vulgaris* or on 5 or more leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution. Dilutions were made with 0.1 M phosphate at pH 7. A dilution of 1-10³ denotes 0.001 gm. of protein per cc., 1-10⁴ denotes 0.0001 gm. of protein per cc., etc.

infectivity on 3 separate globulin preparations. The dilutions are based on the actual amount of globulin present per cc., viz., a dilution of 1-10³ denotes 0.001 gm. of protein per cc., 1-10⁴ denotes 0.0001 gm. of protein per cc., etc. It may be seen that the infectivity of the 3 different crude globulin preparations is about the same. The dark brown globulin fraction, prepared by precipitation and reprecipitation with ammonium sulphate until there was no further loss of color in the filtrate, was found to be infectious at a dilution of 10⁻⁹ gm. per cc. It is referred to as crude globulin and is the starting material that was used for purification.

During the past summer a total of about 4000 kg. of infected fresh Turkish tobacco plants have been processed according to the scheme outlined above. The 4000 kg. of fresh plant material yielded about 5000 liters of extract, in 2 extractions, which contained about 22 kg. of protein before filtration through Hyflo Standard-cel and about 18 kg. of protein after filtration. From this 18 kg. of protein about 14 kg. of once-precipitated globulin was obtained. On reprecipitation a yield of about 80 per cent or 11 kg. of crude globulin was obtained. The globulin fraction thus isolated amounts to about 5.5 per cent of the total solids present in the original extract from

the plant material, or about 0.2 per cent by weight of the extract itself. About $\frac{1}{3}$ of the total nitrogen present in the extract is protein-nitrogen, and of this total about $\frac{1}{2}$ was isolated in the form of the crude globulin-nitrogen.

Purification and Crystallization of the Globulin

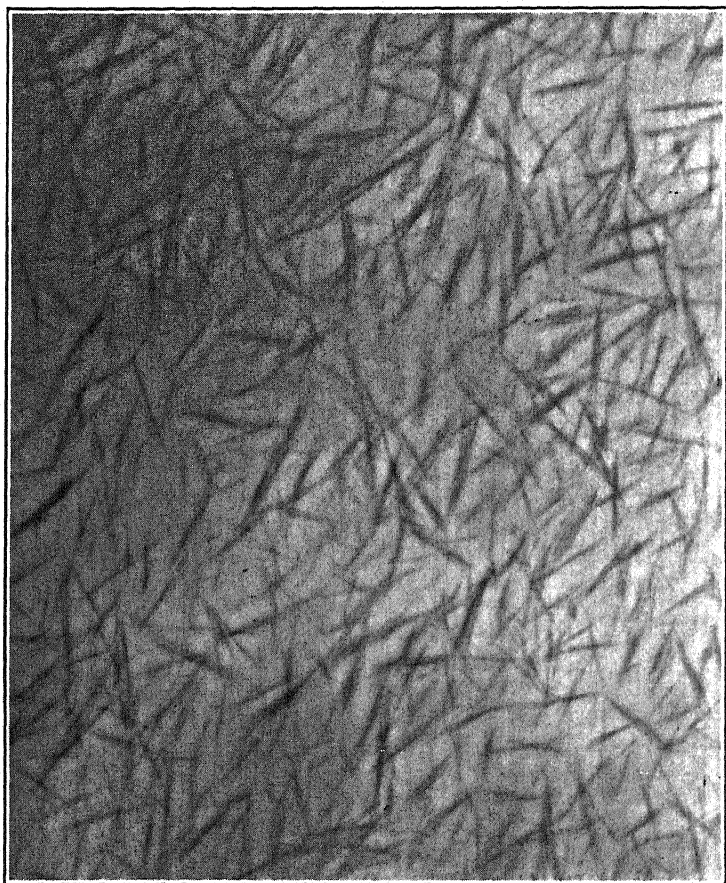
A solution containing 1.2 per cent by weight of crude globulin was adjusted to pH 8.8 with 2N NaOH. Then to 11 different 200 cc. portions of this solution were added varying amounts of a solution containing 200 gm. of lead subacetate per liter. The preparations were then adjusted to about pH 8.8 with 2N NaOH and each filtered through separate Büchner funnels containing a $\frac{1}{4}$ -inch layer of Hyflo Standard-cel. The 11 different filtrates were then adjusted to about pH 7 and protein-nitrogen determinations were made on each sample. The filtrates from preparations to which 1, 2, and 4 cc. of lead subacetate solution had been added were still very dark, but the one to which 6 cc. had been added was definitely lighter in color, and the one to which 8 cc. had been added was yellow and opalescent in appearance. Filtrates from preparations to which 10 or more cc. of lead subacetate solution had been added had an opalescent appearance and varied in color from light yellow to colorless. However, it may be seen from table 4 that, when

TABLE 4.—*Effect of lead subacetate treatment on the protein content and color of a 1.2 per cent solution of active globulin*

Number of cc. of lead subacetate solution added to 200 cc. of a 1.2% protein solution	Total protein in filtrate from lead subacetate precipitate	Per cent protein recovered in filtrate	Color of filtrate
1	2.33	97	Dark brown
2	2.26	94	" "
4	2.29	95	" "
6	1.66	69	Dark yellow
8	1.16	50	Yellow
10	0.35	15	Light yellow
13	0.13	5	Practically colorless
16	0.04	2	" "
20	0.02	1	" "
25	0.03	2	" "
30	0.04	2	" "

10 or more cc. of lead subacetate solution were added to 200 cc. of a 1.2 per cent solution of globulin, only from 1 to 15 per cent of the globulin was recovered in the filtrate. When 6 or 8 cc. of lead subacetate solution were used, most of the color was removed and the filtrate contained from 50 to 70 per cent of the globulin. It is important, therefore, to add no more lead

subacetate than is necessary to precipitate most of the color, since lead subacetate also precipitates the globulin.⁶ About 3 cc. of lead subacetate solution per gram of globulin at a protein concentration of from 0.5 per cent to about 2 per cent has been found satisfactory. The opalescent filtrate from such preparations, which was yellow, was adjusted to about pH 4.5. Two per cent by weight of Hyflo Standard-cel was added, with stirring, and the mixture then filtered through a $\frac{1}{4}$ -inch layer of Filter-cel on a Büchner funnel with suction. The clear yellow filtrate was found to contain no virus and but a small amount of inactive protein, all of the activity having been



Photograph by J. A. Carlile.

FIG. 1. Reproduction of a photomicrograph of crystalline globulin having the properties of tobacco-mosaic virus, which was isolated from infected Turkish tobacco plants. $\times 655$.

⁶ A brief description of an improved method for the purification of crude globulin, in which the use of lead subacetate is omitted, has been published. See *Phytopath.* 26: 108. 1936. (Abst).

adsorbed on the celite. The celite filter cake containing the active protein was then suspended in sufficient water to make a 1 or 2 per cent protein solution. The mixture, which was then adjusted to pH 8 and filtered with suction, gave an opalescent filtrate. The celite filter cake was again suspended in water at pH 8 and filtered in order to remove most of the active protein from the celite. The 2 extracts of the filter cake were combined. Usually 2 or 3 treatments with celite at pH 4.5 and elution at pH 8 were sufficient to yield practically colorless opalescent solutions of active protein.

Crystallization of the globulin in such solutions was accomplished by adding sufficient amounts of a saturated ammonium sulphate solution to cause a faint turbidity and then adding, very slowly, a solution of 5 per cent glacial acetic acid in 0.5 saturated ammonium sulphate, with continual stirring, until the hydrogen-ion concentration was pH 4.5. A solution of saturated ammonium sulphate was then added dropwise until a 20 per cent concentration of ammonium sulphate in the mixture was reached, when crystallization was completed. Figure 1 is a reproduction of a photomicrograph of the crystalline globulin. The crystals are needles about 0.02 mm. long. Smaller crystals may be obtained by causing the protein to come out of solution rapidly. It also was found possible to cause crystallization by adding a little saturated ammonium or magnesium sulphate to a solution of the globulin in 0.001 N sulphuric acid. Although vegetable globulins have been crystallized by dialysis of their solutions, such treatment failed to induce crystallization in the case of the active tobacco-mosaic virus globulin.

General Properties of the Crystalline Material

The crystalline material has the general properties of a protein. A solution containing 1 mg. per cc. gives a positive test with Millon's, biuret, xan-

TABLE 5.—*Elementary analysis and optical activity of crystalline tobacco-mosaic virus protein. Samples dialyzed against dilute HCl, precipitated with acetone, and dried over P₂O₅ in vacuo at 60° C.*

		C	H	N (Dumas)	N (Kjeldahl)	Cl	S	P	Ash
Per cent ash-free dry weight	1 ^a	52.00	7.00	16.09					1.51
		52.38	6.94	16.17					1.77
	2 ^a	53.14	6.90	16.36	16.55	0.50	0.00	0.00	1.49
		53.25	6.99	16.25	16.60	0.52	0.00	0.00	1.30
Optical activity, $[\alpha]_D^{20^\circ}$ per mg. nitrogen. (Samples in dilute NaOH solution at pH 11.2 ± 0.1)							- 0.44		
							- 0.42		

^a Sample No. 1 was dialyzed at pH 4.5 for 4 days, and sample No. 2 was dialyzed at pH 3.25 for 8 days.

thoproteic, glyoxylic acid, and Folin's tyrosine reagents. The material is precipitated from solution by 0.4 saturation with ammonium sulphate, saturation with magnesium sulphate, or by the addition of acetone, ethyl alcohol, or solutions of lead acetate, phosphotungstic acid, safranin, tannic acid, and trichloroacetic acid. The protein is coagulated and the activity lost on heating to 94° C. The analysis and optical rotation of the material are given in table 5. The nitrogen content and optical rotation of the protein were unchanged after it had been subjected to 10 successive crystallizations.

The crystalline material is between 100 and 1000 times more active than ordinary juice preparations from diseased Turkish tobacco plants. The activity might be expected to lie in this range, since 0.2 per cent by weight of the extract from diseased plants was isolated in the form of crystals. Table 6 gives the results of several activity tests on the first crystalline protein that was obtained, on the same after 10 successive crystallizations, and on a sample of infectious juice pressed from fresh macerated plants. Most of the activity measurements were made by the half-leaf method, using plants of *Phaseolus vulgaris* L., *Nicotiana glutinosa*, and *N. langsdorffii* Schrank. Dilutions of a solution of the crystalline material were made with 0.1M phosphate at pH 7. The undiluted infectious juice preparation was compared with a 10^{-8} dilution or 1 mg. per cc. of the crystals. It may be seen that the crystalline material is slightly less than 1000 times as active as the infectious juice. It also may be seen that the crystalline protein was active at a dilution of 10^{-9} gm. per cc., and that in several tests it was impossible to detect a significant difference between the activity of protein crystallized once and the activity of protein crystallized 10 times. The disease produced in healthy Turkish tobacco plants on inoculation with suspensions of the lesions caused by a solution containing 10^{-9} or more grams of crystalline protein per cc. has always proved to be typical tobacco mosaic. Large amounts of virus were found in plants inoculated with a suspension from 1 lesion from a leaf of *P. vulgaris* previously inoculated with a solution containing 10^{-9} gm. per cc. of the crystals.

The crystalline material is practically insoluble in water, although readily soluble in dilute solutions of acids, alkalis, or salts. Most of the work on the crystalline material was done with solutions containing about 1 per cent protein by weight. Solutions containing from 0.1 per cent to 2 per cent protein have a decided opalescent appearance. Such solutions are fairly clear between pH 6 and 11, but below about pH 5 take on a dense whitish appearance which becomes very pronounced at about pH 3.2. If solutions of the protein are made more alkaline than about pH 11, the opalescence disappears and they become clear. However, such solutions were inactive and it was found by solubility tests that the protein had been denatured. The material is also denatured and the activity lost when solutions are made more

TABLE 6.—*Infectivity tests on crystalline globulin and on infectious juice*

	Test plant	No. of half-leaves	Method ^a	1-10 ^{3b}	1-10 ⁴	1-10 ⁵	1-10 ⁶	1-10 ⁷	1-10 ⁸	1-10 ⁹
1x crystallized 10x crystallized	P. vulgaris	12 12	L R	139.5 ^c 178.1	43.6 32.0	16.3 25.8	1.5 1.6	0.2 0.4	0.0 0.1	0.0 0.0
1x crystallized 10x crystallized	N. glutinosa	10 10	L R	67.1 98.0	65.0 44.0	14.2 19.9	4.4 3.2	0.9 0.6	0.1 0.2	0.0 0.1
1x crystallized 10x crystallized	N. langsdorffii	5 5	L R	179.0 213.0	160.0 119.0	86.0 60.0	9.4 12.3			
10x crystallized 1x crystallized	P. vulgaris	12 12	L R	95.5 83.1	49.9 49.1	2.5 7.6	2.3 8.0	0.6 0.7	0.2 0.2	0.0 0.1
1x crystallized 10x crystallized	P. vulgaris	180 180	P P			19.5 21.8				
Crystals	P. vulgaris	24	W	131.9	99.3	13.4	2.5	0.7	0.2	0.1
Crystals Infectious juice	P. vulgaris	24 12	W L	51.6 187.5	45.5 66.5	10.5 42.1	1.6 18.5	0.4 4.1	0.1 1.6	0.0 0.4
Crystals Infectious juice	N. glutinosa	10 10	L R	22.4 21.0	26.2 31.8	22.7 11.7	2.5 1.5	1.0 0.7	0.1 0.1	1.4

^a L indicates inoculation was on left leaf-halves, R right leaf-halves of same leaves. P indicates one whole leaf of each plant was inoculated with 1x crystallized material and the other whole leaf of the same plant was inoculated with 10x crystallized material. W indicates that the whole leaf was inoculated.

^b All dilutions were made with phosphate at pH 7. Designated dilutions of infectious juice should be divided by 1000. Undiluted juice was compared with a 1-10³ dilution of crystals and then both preparations were diluted equally. A 1-10³ dilution of crystals denotes a solution containing 0.001 gm. of protein per cc., 1-10⁴ denotes 0.0001 gm. of protein per cc., etc.

^c Numbers represent the average number of lesions per half-leaf obtained on inoculation with the designated preparation and dilution.

acid than about pH 1. The active protein readily passes a Berkefeld "W" filter, but fails to pass collodion filters through which proteins, such as egg albumin, readily pass. It has not been found possible to separate the activity from the protein by means of collodion filters.

Table 7 records the results of a number of precipitin tests made with

TABLE 7.—*Results of precipitin tests with crystalline protein*

Dilution	Anti-crystalline protein guinea-pig serum	Normal guinea-pig serum	Anti-infectious tobacco juice rabbit serum	Normal rabbit serum	Anti-normal tobacco juice rabbit serum	Normal rabbit serum
Crystalline protein						
1-1,000	++++ ^a	-	+++	-	-	-
1-10,000	++	-	++	-	-	-
1-50,000	±	-	+	-	-	-
1-100,000	-	-	±	-	-	-
1-200,000	-	-	-	-	-	-
Infectious juice from Turkish tobacco plants						
1	++	-	+++	-	-	-
1-5	+	-	+	±	-	-
1-10	-	-	+	-	-	-
1-50	-	-	±	-	-	-
1-100	-	-	-	-	-	-
Juice from normal Turkish tobacco plants						
1	-	-	-	-	+	-
1-5	-	-	-	-	±	-
1-10	-	-	-	-	±	-
1-50	-	-	-	-	-	-
1-100	-	-	-	-	-	-

^a++++ indicates a very heavy precipitate was obtained.

++ indicates a heavy precipitate was obtained.

++ indicates a moderate precipitate was obtained.

+ indicates a slight precipitate was obtained.

± indicates a very slight precipitate was obtained.

- indicates no precipitate was obtained.

sera kindly prepared by Dr. K. S. Chester. It may be seen that the serum of an animal injected with a solution of the crystalline material gives a precipitate when mixed with infectious juice or with solutions of the crystals, and fails to give a reaction with the juice from normal tobacco plants. The sera of animals injected with infectious juice give a precipitate when mixed with a solution containing but 1 part in 100,000 of the crystalline protein, whereas the sera of animals injected with juice from normal tobacco plants

give no precipitate when mixed with a solution of the crystals. The fact that a solution of the crystals may be diluted so high and yet give a visible precipitate when mixed with immune serum indicates that the crystalline material is essentially pure and free from foreign material. The failure to obtain a precipitate when serum from animals injected with crystalline protein is mixed with an extract from normal plants, or when serum from animals injected with extracts from normal plants is mixed with a solution of crystalline material, is strong evidence that the active crystallizable protein does not exist in normal plants.

DISCUSSION

The isolation of a highly infectious crystalline protein from extracts of Turkish tobacco plants diseased with tobacco-mosaic virus brings to the forefront many problems having to do with viruses. Ever since 1898, when Beijerinck (2) introduced his theory of a contagious living fluid, much discussion has centered around the question as to whether or not viruses are alive. The evidence presented in this paper indicates that the virus of tobacco mosaic, the first to be described in the literature (7), is not alive. It is exceedingly difficult to conceive of a living agent that may be not only crystallized, but recrystallized repeatedly, with retention of constant physical, chemical, and biological properties. Such characteristics are regarded as those of pure chemical compounds. Hence it seems reasonable to assume that the crystalline protein is a chemical compound and, therefore, inanimate. The question then arises as to whether the virus activity is a property of the protein molecule, or is a property of something, living or nonliving, adsorbed on the protein. If a living agent is responsible for the activity, then, in view of the constant properties that the crystalline protein possesses, it certainly is not an ordinary contaminant. It may be possible that some hitherto unknown close association may exist between a crystalline protein and a living agent. On the basis of present knowledge, such an association seems very unlikely and it is mentioned only as a possibility. If a nonliving contaminant exists in the crystals, then it follows, of course, that the virus itself is nonliving. However, no evidence for the existence of a mixture of active and inactive material in the crystals has been obtained. Although it is difficult, if not impossible, to obtain conclusive positive proof of the purity of a protein, all of the evidence obtained up to the present time indicates that the crystalline material is pure. Since it is of great importance to know with as much certainty as possible whether or not the crystalline protein is pure, experiments from which it is hoped further evidence on this point will be obtained are now in progress.

The fact that this virus seems to be a protein may necessitate a fundamental change in the conception of the nature of other viruses. That a

chemical substance should possess properties that endow it with the disease-producing potentialities of tobacco-mosaic virus is most striking. Yet, when examined in the light of recent developments in protein chemistry, the phenomenon is not so surprising. A crystalline protein, trypsinogen, recently isolated by Kunitz and Northrop (10), is distinguished by its lack of hydrolytic properties. However, on addition of a little trypsin the inactive trypsinogen is converted into trypsin, an active hydrolytic agent. The activation reaction is autocatalytic and may be demonstrated *in vitro*. The tobacco-mosaic virus protein is somewhat different in that the presence of living cells appears to be necessary for multiplication of the protein. If the precursor of the virus protein should be an unstable or transient form of protein, existent only in association with the living cells of certain plants, then it will be possible to demonstrate catalysis or multiplication of virus only in the presence of such living cells. If, on the other hand, the precursor should be stable, then it should be possible to catalyze the production of virus protein *in vitro* by the addition of a little of the active protein to the precursor. Furthermore, it is conceivable that the precursor might exist in some plants and fail to exist in other plants. The latter group would be immune from the tobacco-mosaic disease, since virus protein would be expected to multiply only in hosts having the precursor of the active protein introduced. This may be the explanation of the host specificity of tobacco-mosaic virus.

On the basis that this virus is a protein, a chemical explanation of infection as the inauguration of an autocatalytic reaction and of the disease itself as a resultant of the disruption of normal protein metabolism caused by the production of an enormous amount of foreign protein appears feasible. The actual isolation of over 2 mg. of active protein per cubic centimeter of plant extract demonstrates that it is present in the plant material in relatively large amounts. The production in such large quantities brings up the question of how the virus protein spreads in plants. It seems likely that it moves rapidly in the phloem vessels (12). The cell-to-cell type of virus movement, which has been so difficult to account for in the past, might be readily explained if one assumes that active protein in one cell catalyzes the production of protein in adjacent cells without passing from cell to cell. Thus, although any one molecule might not move out of the cell in which it is produced, the result of such catalysis would be an apparent rapid movement due to contact of active protein at the periphery of one cell on inactive protein in adjacent cells. It is likely that diffusion and protoplasmic streaming play a part in the movement of active protein molecules, but it is difficult to account for the movement of such large molecules by diffusion and protoplasmic streaming alone. The nature of the intracellular inclusions found in plants infected with tobacco mosaic remains to be determined.

The bodies may consist of active protein, or of aggregates of essentially inactive protein separated during the production of virus protein.

The occurrence of many strains (8) of tobacco-mosaic virus has a fairly logical explanation on a chemical basis, since they may result from changes in the active molecule to give similar or isomeric proteins. It is well known that slightly unstable molecules may, under certain conditions, undergo rearrangement or polymerization to give molecules having different physical and chemical properties. In a molecule as large as the active protein, many different changes, giving rise to different molecules and hence to many different virus strains, might be expected. Such change in the structure of an active protein molecule might be regarded as an example of adaptation.

The isolation of a typical virus in the form of a crystalline protein permits many phases of the general virus problem to be viewed with a new significance. Although some workers hold that certain viruses are living agents, it appears reasonable to assume that a considerable number of them may be proteins similar in nature to the virus of tobacco mosaic. The multiplication, adaptation, and specificity of viruses, their possible dependence on the presence of living cells for multiplication, and even immunity, may be explained on a chemical basis without great difficulty.

SUMMARY

A crystalline protein, which has the properties of tobacco-mosaic virus, has been isolated from an extract of Turkish tobacco plants infected with this virus. The extract was prepared by grinding frozen plants, adding disodium phosphate, and pressing out the liquid. The press cake was extracted a second time with dilute disodium phosphate and the 2 extracts were filtered through celite and combined. The virus protein was obtained from these extracts by precipitation with ammonium sulphate. The virus protein was reprecipitated with ammonium sulphate several times with loss of much color, and most of the remaining color was then removed with lead subacetate. The virus was adsorbed on, and removed from, celite several times and then crystallized in the form of small needles about 0.02 mm. long by the addition of a solution of 5 per cent glacial acetic acid in 0.5 saturated ammonium sulphate.

The crystalline material has the general properties of a protein and its infectivity, chemical composition, and optical rotation were unchanged after 10 crystallizations. The material is between 100 and 1000 times more active than ordinary infectious juice preparations, one cubic centimeter of a solution containing 10^{-9} gm. per cc. of the protein usually proving infectious. The crystalline material reacts with sera of animals injected with a solution of the crystals or with infectious juice, and fails to react with the sera of animals injected with juice from normal plants. All of the evidence obtained

up to the present time indicates that the crystalline protein is pure or possibly a solid solution of proteins, and hence, that tobacco-mosaic virus is a protein.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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SEED-POTATO TREATMENT FOR RHIZOCTONIA CONTROL IN NORTHEASTERN MAINE, 1929 TO 1933¹

W. P. RALEIGH² AND REINER BONDE³

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INTRODUCTION

The results of seed-potato treatment experiments in northeastern Maine for the years 1925 to 1928 were published by Schultz, Gratz, and Bonde (7). The experiments in the same locality for the years 1929 to 1933 were conducted in order to test the effectiveness of commercial products against rhizoctonia and, if possible, to develop new and more effective short-time treatments. The problem of soil infestation was given some consideration and information was obtained on the value of selection of clean seed potatoes for planting.

METHODS

In general, the methods used in these experiments were similar to those used by Schultz, Gratz, and Bonde (7). Three kinds of seed potatoes were used: Rhizoctonia-infected, clean, and field-run.

Rhizoctonia-infected tubers were used in order to get an index of the effectiveness of the treatments. They were selected at harvest time and only those with a high percentage of their surface covered by rhizoctonia sclerotia were taken. Most of the sclerotia were less than $\frac{1}{16}$ inch thick, which is typical of the rhizoctonia black-scurf stage of infection in northeastern Maine. All of the rhizoctonia-infected tubers used in these experiments were of the Irish Cobbler variety.

The clean tubers were used to give an index of possible injurious effects of the treatments and to give an index of soil infestation by rhizoctonia. Only tubers that showed no rhizoctonia sclerotia when washed or brushed were used as clean seed. It is probable that some of these tubers were lightly infected by rhizoctonia and escaped notice. In all experiments on rhizoctonia-infected seed a nontreated clean check and a treated clean check, as used by Melhus and Gilman (4), were included. From 1930 to 1933 separate experiments were conducted in which only clean tubers were used. This method largely avoids the possibility of field spread from the diseased rows to the clean ones and therefore gives a good index of soil infestation. Clean, 1½-ounce whole tubers were included in the clean Irish

¹ Conducted as a cooperative project between the Bureau of Plant Industry, U. S. Department of Agriculture, and the Maine Agricultural Experiment Station.

² Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

³ Associate Plant Pathologist, Maine Agricultural Experiment Station.

Cobbler experiments in 1931, 1932, and 1933 as a possible check on seed-piece decay.

The field-run tubers were used in order to secure data on the value of treatment of what might be considered average commercial Green Mountains in northeastern Maine. They were graded and only primes were used for seed, but no selection regarding rhizoctonia was made. They were grown from treated tubers and usually bore rhizoctonia sclerotia on less than 10 per cent of the tubers. In general, the Green Mountain potatoes grown in Maine are not so heavily infected with rhizoctonia as are the Irish Cobblers.

The seed tubers, with the exception of a few cases where they were treated in the fall, were planted shortly after being treated. This interval varied from 5 to 19 days and was usually less than 2 weeks. All treatments were made before the tubers were cut.

The treatments varied in length from dip treatments to 2-hour soaks. By a dip treatment is meant that the tubers either were dipped 3 times in rapid succession into a solution or were placed in a solution, agitated slightly, and then removed from the solution. By either of these methods the tubers are more thoroughly wetted than if they are merely dipped once into a solution, especially if they are covered with a film of dust. After treatment the tubers were placed in bushel crates that allowed the passage of air and aided in drying them.

Throughout the 5-year period, all tubers, except those planted whole, were cut by one man to as nearly 1½-ounce seed pieces as possible.

Only seed tubers from a common source were included in each experiment.

In all cases 10 plots of 28 hills each were planted for yield. Just before harvest the end hills of these plots were removed, leaving 26 hills. The plots for yield were arranged according to the chessboard plan, a modification of the magic-square system, as employed by Schultz, Gratz, and Bonde (7) in 1927 and 1928. Two extra plots for examination for stem lesions and seed-piece decay were planted outside the buffer rows of each experiment.

All plots were planted on Aroostock Farm, Presque Isle, Maine, on land that was in a 3-year rotation of oats, clover, and potatoes. The most uniform land available was selected and commercial fertilizer was applied, at the rate of about 2000 pounds per acre, in the rows with a planter that mixed it with the soil. The date of planting varied from May 11 to May 23.

All seed pieces were dropped by hand at 14-inch intervals in rows approximately 3 feet apart. The seed pieces were covered with a horse-hoe. In the years 1929, 1930, and 1931 they were covered to a depth of about 6 inches, while in 1932 and 1933 they were covered to a depth of about 4½ inches.

The plants in the extra rows usually were about 4 inches tall when they

were dug and examined for seed-piece decay and stem lesions. Since only a very few decayed seed pieces were found in any of the experiments, the data on such decay are omitted from the tables.

Notes were taken on stand and vigor usually when the plants were from 6 to 10 inches tall. Regarding vigor, the height of each plant was estimated and the relative vigor, as recorded in the tables, was determined from these estimates.

All plots were cultivated and sprayed as uniformly as possible. Considerable variation in injury to the different plots was encountered in 1930 with the use of a tractor in spraying.

The plots were harvested soon after the plants had died. The tubers were graded according to the standards of the United States Department of Agriculture and figures were obtained on primes, seconds, and culls. For yield comparisons, only primes and seconds were used and these are referred to as marketable tubers. Except in the experiments where clean tubers were planted and the potatoes harvested had only a very low percentage of infection, 100 tubers from each plot were taken at random, washed, and examined for rhizoctonia sclerotia. This made 1,000 tubers for each treatment. All tubers showing any rhizoctonia sclerotia were classed as infected. According to the work of Bisby, Higham, and Groh (1), it is probable that rhizoctonia infection might have been heavier had the plots been harvested later.

$$\text{The formulae PEM} = \frac{.675 \times \text{standard deviation}}{\sqrt{\text{No. of items}}}$$

$$\text{and PED} = \sqrt{a^2 + b^2}, \text{ where}$$

a = probable error of the mean of the treated lot and b = probable error of the check with which the treated lot is compared, were used for determining the significance of the differences in yield. Odds as listed by Pearl and Miner (5) were used.

1929 EXPERIMENTS

The results of experiments conducted in 1929 are given in tables 1 and 2. Nine treatments were compared with an untreated check on rhizoctonia-infected and clean Irish Cobblers of a common origin and included in one experiment. The same 9 treatments were used on field-run Green Mountains in comparison with a nontreated check and with a treated and a nontreated lot of clean Green Mountains from the same source. The treatments used are listed in tables 1 and 2. The last 3 materials listed in table 1 were experimental products furnished by the Bayer-Semesan Company.

Of the treatments used, K 8 was the most injurious, as indicated by the relative vigor of the plants from seed treated with this material. Clean, nontreated Irish Cobblers showed a relative vigor of 96, while those treated

TABLE 1.—Seed-potato treatment results—Irish Cobblers 1929

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over diseased nontreated check	Approximate odds
Diseased, check, not treated	99.6	84	71	79	261.7 ± 5.5	47.8 ± 6.7	434,782:1
Diseased, corrosive sublimate 1-1,000	99.2	95	2	13	309.5 ± 3.9	32.3 ± 6.6	1,052:1
Diseased, corrosive sublimate 1-500	99.6	95	10	20	294.0 ± 3.7		
Diseased, corrosive sublimate 1-500 and 0.8% glacial acetic acid	99.2	96	4	8	305.2 ± 4.8	43.5 ± 7.3	19,230:1
Diseased, formaldehyde 1-120, 52° C.	98.5	92	16	28	289.6 ± 6.6	27.9 ± 8.6	31:1
Diseased, formaldehyde 1-120, 11° C.	98.8	94	12	14	309.1 ± 6.5	47.4 ± 8.5	19,230:1
Diseased, Semesan Bel 1-20	99.2	93	29	52	298.0 ± 4.5	36.3 ± 7.1	1,350:1
Diseased, Cal K 1-40	99.6	92	2	5	301.6 ± 6.0	39.9 ± 8.1	1,052:1
Diseased, K 8 1-40	96.5	77	0	3	269.3 ± 4.5	7.6 ± 7.1	1:1
Diseased, Dubay No. 664 1-68	99.6	96	4	9	305.6 ± 5.2	43.9 ± 7.6	19,230:1
Clean, check, not treated	100	96	2	2	312.3 ± 5.6	50.6 ± 7.8	19,230:1
Clean, corrosive sublimate 1-1000	99.6	95	2	1	301.6 ± 6.1	39.9 ± 8.2	1,052:1
Clean, corrosive sublimate 1-500	98.8	96	0	3	310.3 ± 4.0	48.6 ± 6.8	434,782:1
Clean, corrosive sublimate 1-500 and 0.8% glacial acetic acid	100	97	0	3	308.7 ± 5.5	47.0 ± 7.8	19,230:1
Clean, formaldehyde 1-120, 52° C.	98.8	98	0	2	313.5 ± 5.5	51.8 ± 7.8	434,782:1
Clean, formaldehyde 1-120, 11° C.	99.6	96	2	1	298.4 ± 3.3	36.7 ± 6.4	19,230:1
Clean, Semesan Bel 1-20	100	96	0	2	316.7 ± 5.8	55.0 ± 8.0	434,782:1
Clean Cal K 1-40	100	92	2	3	293.6 ± 5.4	31.9 ± 7.7	175:1
Clean K 8 1-40	97.7	78	0	2	266.5 ± 5.2	4.8 ± 7.6	0
Clean Dubay No. 664 1-68	99.2	96	0	1	309.5 ± 6.1	47.8 ± 8.2	19,230:1

TABLE 2.—Seed-potato treatment results—Green Mountains 1929

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over field-run nontreated check	
						Bushels	Approximate odds
Field-run, check, not treated	99.2	99	23	6	318.7 ± 5.6
Field-run, corrosive sublimate 1-1000 1½ hrs.	99.2	99	10	1	327.5 ± 5.5	8.8 ± 7.8	1:1
Field-run, corrosive sublimate 1-500 3 min.	99.6	99	12	4	321.1 ± 6.3	2.4 ± 8.4	0
Field-run, corrosive sublimate 1-500 and .8% glacial acetic acid	96.9	98	2	1	316.3 ± 8.1	-2.4 ± 9.8	0
Field-run, formaldehyde 1-120, 52° C. 3 min.	96.9	97	8	6	301.9 ± 5.1	-16.8 ± 7.6	6:1
Field-run, formaldehyde 1-120, 11° C. 3 min.	100	98	6	2	320.3 ± 6.0	1.6 ± 8.2	0
Field-run, Semesan Bel 1-20 1 min.	98.8	98	17	8	321.1 ± 5.5	2.4 ± 7.8	0
Field-run, Cal K 1-40 1 min.	99.2	98	2	1	314.7 ± 6.6	-4.0 ± 8.7	0
Field-run, K 8 1-40 1 min.	98.8	95	5	1	300.8 ± 5.6	-17.9 ± 7.9	7:1
Field-run, Dubay No. 664 1-63 1 min.	99.2	98	4	2	315.1 ± 7.4	-3.6 ± 9.3	0
Clean, check, not treated	100	99	6	3	319.5 ± 8.5	0.8 ± 10.2	0
Clean, corrosive sublimate 1-1000 1½ hrs.	99.2	100	8	1	321.5 ± 5.2	2.8 ± 7.6	0

TABLE 3.—Seed-potato treatment results—Irish Cobblers 1930

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over diseased nontreated check	Approx- imate odds
Diseased, check, not treated	98.8	85	88	82	402.5 ± 8.8
Diseased, corrosive sublimate 1-1000	100	94	15	11	439.6 ± 12.8	37.1 ± 15.5	8: 1
Diseased, corrosive sublimate 1-500	99.2	88	23	52	432.0 ± 6.3	29.5 ± 10.8	14: 1
Diseased, corrosive sublimate 1-500 and .36% glacial acetic acid	100	92	10	16	442.4 ± 6.2	39.9 ± 10.8	78: 1
Diseased, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	100	93	12	10	450.4 ± 8.0	47.9 ± 11.9	142: 1
Diseased, formaldehyde 1-120, 52° C. covered 1 hr.	100	94	10	34	452.4 ± 9.1	49.9 ± 12.7	116: 1
Diseased, formaldehyde 1-120, 10° C. covered 1 hr.	99.6	88	56	70	423.6 ± 9.3	21.1 ± 12.8	3: 1
Diseased, formaldehyde 1-240	99.6	87	48	66	414.5 ± 6.0	12.0 ± 10.7	1: 1
Diseased, Dubay No. 664 1-64	99.6	93	35	35	440.0 ± 11.3	37.5 ± 14.3	12: 1
Diseased, Dubay No. 693 1-64	99.6	92	6	17	448.8 ± 10.7	46.3 ± 13.9	37: 1
Diseased, Dubay No. 738 1-80	97.3	82	2	8	433.6 ± 8.0	31.1 ± 11.9	12: 1
Diseased, Sanosced 1-60	99.6	95	10	15	441.6 ± 11.5	39.1 ± 14.5	14: 1
Clean, check, not treated	99.2	95	6	2	429.2 ± 11.4	26.7 ± 14.4	4: 1
Clean, corrosive sublimate 1-1000	99.2	93	2	4	435.2 ± 9.7	32.7 ± 13.1	10: 1

TABLE 4.—Seed-potato treatment results—clean Irish Cobblers 1930

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over nontreated check	
						Bushels	Approximate odds
Check, not treated	100	96	2	1	438.8 ± 9.4
Corrosive sublimate 1-1000	99.6	93	0		453.9 ± 6.6	15.1 ± 11.5	2: 1
Corrosive sublimate 1-500	99.6	94	0		457.9 ± 8.8	19.1 ± 12.9	2: 1
Corrosive sublimate 1-500 and .56% glacial acetic acid	100	95	0		461.9 ± 7.5	23.1 ± 12.0	4: 1
Corrosive sublimate 1-500 and 1% commercial hydrochloric acid	100	95	0		448.0 ± 7.8	9.2 ± 12.2	0
Formaldehyde 1-120, 52° C. 3 min., covered 1 hr.	99.6	95	0		441.2 ± 6.6	2.4 ± 11.5	0
Formaldehyde 1-120, 10° C. 3 min., covered 1 hr.	100	95	0		435.2 ± 6.5	- 3.6 ± 11.4	0
Formaldehyde 1-240	100	96	0		437.6 ± 12.8	- 1.2 ± 15.9	0
Dubay No. 664 1-64	100	95	0		463.5 ± 8.2	24.7 ± 12.5	5: 1
Dubay No. 693 1-64	100	94	0		453.2 ± 7.8	14.4 ± 12.2	1: 1
Dubay No. 738 1-80	98.8	85	0		421.6 ± 7.7	- 17.2 ± 12.2	2: 1
Sanoseed 1-60	100	93	0		439.6 ± 6.9	0.8 ± 11.7	0

TABLE 5.—Seed potato treatment results—Green Mountains 1980

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Bushels	Increase over field-run nontreated check Approximate odds
Field-run, check, not treated	99.6	98	21	13	434.4 ± 9.8
Field-run, corrosive sublimate 1-1000	99.2	99	10		463.5 ± 11.6	29.1 ± 15.2	4: 1
Field-run, corrosive sublimate 1-500	100	98	8		443.6 ± 8.3	9.2 ± 12.8	0
Field-run, corrosive sublimate 1-500 and .56% glacial acetic acid	100	99	2		446.4 ± 5.7	12.0 ± 11.3	1: 1
Field-run, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	99.6	97	0		430.8 ± 8.5	-3.6 ± 13.0	0
Field-run, formaldehyde 1-120, 52° C. 3 min., covered 1 hr.	100	99	2		461.9 ± 8.9	27.5 ± 13.2	5: 1
Field-run, formaldehyde 1-120, 10° C. 3 min., covered 1 hr.	98.5	98	6		432.0 ± 7.8	-2.4 ± 12.5	0
Field-run, formaldehyde 1-240 2 hrs.	99.2	98	4		415.7 ± 8.9	-18.7 ± 13.2	2: 1
Field-run, Dubay No. 664 1-64 dip	99.6	98	25		429.6 ± 9.9	-4.8 ± 13.9	0
Field-run, Dubay No. 693 1-64 dip	98.5	98	4		434.0 ± 8.8	-0.4 ± 13.2	0
Field-run, Dubay No. 738 1-80 dip	100	95	0		448.4 ± 8.6	14.0 ± 13.0	1: 1
Field-run, Sanoseed 1-60 dip	100	98	0		445.2 ± 7.4	10.8 ± 12.3	0
Clean, check, not treated	100	99	4	7	454.7 ± 9.8	20.3 ± 13.9	2: 1
Clean, corrosive sublimate 1-1000 1½ hrs.	99.2	98	0		426.4 ± 9.9	-8.0 ± 13.9	0

with K 8 showed a relative vigor of 78. Also, the yield of the clean Irish Cobblers was much lower with K 8 than with any other treatment.

With the exception of K 8, all of the treatments on the rhizoctonia-infected seed gave significant increases in yield ranging from 27.9 to 47.8 bushels per acre.

Semesan Bel was decidedly inferior in its control of Rhizoctonia. For hot formaldehyde also there was 28 per cent tuber infection compared with 13 per cent for the standard corrosive sublimate. In these tests the usual 1-hour covering following the hot formaldehyde treatment was omitted; therefore, these results cannot be accepted as representative of the regular hot-formaldehyde treatment.

Only 2 of the treated lots of clean Irish Cobblers yielded more than the nontreated check, and these differences were not significant.

On the field-run Green Mountains most of the treatments decreased the number of stem lesions and the amount of tuber infection, but there was no significant difference between the yield from the nontreated check and that from any of the treated lots.

1930 EXPERIMENTS

The 1930 experiments were similar to those conducted in 1929 except that clean Irish Cobblers were planted in a separate experiment. The results are recorded in tables 3, 4, and 5. The 3 numbered materials listed in the tables under the name Dubay were experimental products furnished by the Bayer-Semesan Company.

The acid corrosive sublimate treatment (corrosive sublimate 1-500 with 1 per cent commercial hydrochloric acid) was the same as that used by Leach, Johnson, and Parson (3), except that the time of treatment was 3 minutes instead of 5.

The strength of the glacial acetic acid was changed from the 0.8 per cent used in 1929 to 0.56 per cent, since that strength gave about the same acidity as determined by titrating against an alkali as 1 per cent commercial hydrochloric acid as used in the regular acidulated corrosive sublimate treatment.

The experimental product Dubay No. 664 did not control rhizoctonia so well as in 1929. According to information received from the company, the 1930 product was made by a different process than the 1929 product and apparently was not identical with it.

All treatments used on Rhizoctonia-infected tubers gave increased yields, but the increases were statistically significant in only 4 instances.

None of the treatments caused a significant difference in yield of either the clean Irish Cobblers or the field-run Green Mountains.

In 1930 the plots were sprayed with a tractor, which caused marked injury in some instances and accounted for part of the variability recorded.

TABLE 6.—Seed-potato treatment results—Irish Cobbler 1931

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over diseased nontreated check	
						Bushels	Approximate odds
Diseased, check, not treated	99.6	84	71	60	321.1 ± 11.5
Diseased, corrosive sublimate 1-1000	99.2	95	8	26	338.7 ± 10.8	17.6 ± 15.8	1:1
Diseased, corrosive sublimate 1-500	100	94	40	43	351.4 ± 11.0	30.3 ± 15.9	4:1
Diseased, corrosive sublimate 1-500 and .56% glacial acetic acid	99.2	93	37	31	333.9 ± 9.8	12.8 ± 15.1	0
Diseased, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	97.7	96	10	25	354.6 ± 11.9	33.5 ± 16.5	5:1
Diseased, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	98.5	94	29	36	331.1 ± 11.2	10.0 ± 16.1	0
Diseased, formaldehyde 1-120, 52° C. 3 min., covered 1 hr.	97.3	85	38	34	345.4 ± 9.9	24.3 ± 15.2	3:1
Diseased, formaldehyde 1-120, 8° C. 3 min., covered 1 hr.	98.8	85	63	57	314.3 ± 10.1	-6.8 ± 15.3	0
Diseased, formaldehyde 1-240 2 hrs.	100	92	48	35	365.4 ± 6.1	44.3 ± 13.0	45:1
Diseased, New Improved Semesan Bel 1-60 dip	99.6	95	35	36	341.5 ± 13.2	20.4 ± 17.5	1:1
Diseased, Sanoseed 1-60 dip	99.6	97	23	27	356.2 ± 6.1	35.1 ± 13.0	14:1
Diseased, yellow mercuric oxide 1-100 dip	100	88	0	11	322.3 ± 9.7	1.2 ± 15.0	0
Diseased, corrosive sublimate 1-1200 and potassium iodide 1-400 dip	100	96	10	31	367.0 ± 8.3	45.9 ± 14.2	31:1
Diseased, corrosive sublimate 1-500 and ammonium carbonate 1-40 3 min.	98.8	96	21	29	338.3 ± 10.0	17.2 ± 15.3	1:1
Diseased, corrosive sublimate 1-1000 treated 9/22/30	100	96	15	28	364.2 ± 9.8	43.1 ± 15.1	19:1
Clean, check, not treated	100	97	8	16	360.6 ± 10.3	39.5 ± 15.4	12:1
Clean, corrosive sublimate 1-1000 1½ hrs.	100	96	4	13	350.6 ± 10.7	29.5 ± 15.7	4:1

TABLE 7.—Seed-potato treatment results—clean Irish Cobblers 1931

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over non- treated check	Approx- imate odds
Check, not treated	99.2	99	0	1	399.7 ± 8.1
Corrosive sublimate 1-1000	98.1	98	4	1	401.3 ± 8.5	1.6 ± 11.7	0
Corrosive sublimate 1-500	100	98	2		402.9 ± 8.5	3.2 ± 11.7	0
Corrosive sublimate 1-500 and .56% glacial acetic acid	97.3	97	2		406.1 ± 7.3	6.4 ± 10.9	0
Corrosive sublimate 1-500 and 1% commercial hydrochloric acid	98.8	98	2		411.7 ± 10.8	12.0 ± 13.5	0
Corrosive sublimate 1-500 and 1% commercial hydrochloric acid	98.1	98	2		411.3 ± 6.8	11.6 ± 10.6	1:1
Formaldehyde 1-120, 52° C. 3 min., covered 1 hr.	93.1	94	2		386.9 ± 9.8	-12.8 ± 12.7	1:1
Formaldehyde 1-120, 8° C. 3 min., covered 1 hr.	95.8	98	0		397.7 ± 6.4	-2.0 ± 10.3	0
Formaldehyde 1-240	97.3	98	2		404.9 ± 9.9	5.2 ± 12.8	0
New Improved Semesan Bel 1-60	99.6	98	0		398.1 ± 8.0	-1.6 ± 11.4	0
Sanoseed 1-60	98.1	99	2		396.9 ± 7.1	-2.8 ± 10.8	0
Yellow mercuric oxide 1-100	98.8	95	0		388.9 ± 8.4	-10.8 ± 11.7	0
Corrosive sublimate 1-1200 and potassium iodide 1-400	99.2	98	6		402.1 ± 7.8	2.4 ± 11.2	0
Corrosive sublimate 1-500 and ammonium carbonate 1-40	99.2	98	0		394.5 ± 7.5	-5.2 ± 11.0	0
1½-oz. whole tubers, check, not treated	98.8	100	2		435.2 ± 9.3	35.5 ± 12.3	19:1
1½-oz. whole tubers, corrosive sublimate 1-1000	99.6	99	4		413.3 ± 7.1	13.6 ± 10.8	2:1

TABLE 8.—Seed-potato treatment results—Green Mountains 1931

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over field-run nontreated check	Approximate odds
Field-run, check, not treated	100	99	4	2	431.6 ± 8.6
Field-run, corrosive sublimate 1-1000	100	99	0	3	426.8 ± 9.3	-4.8 ± 12.7	0
Field-run, corrosive sublimate 1-500	99.6	99	0		416.5 ± 6.9	-15.1 ± 11.0	2:1
Field-run, corrosive sublimate 1-500 and 56% glacial acetic acid	98.8	99	0		409.3 ± 7.9	-22.3 ± 11.7	4:1
Field-run, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	97.3	97	0		417.6 ± 7.0	-14.0 ± 11.1	2:1
Field-run, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	99.6	98	0		434.4 ± 8.7	2.8 ± 12.2	0
Field-run, formaldehyde 1-120, 52° C.	93.5	91	0		388.5 ± 6.6	-43.1 ± 10.8	142:1
Field-run, formaldehyde 1-120, 8° C.	98.8	98	8		425.6 ± 9.8	-6.0 ± 13.0	0
Field-run, formaldehyde 1-240	99.6	99	0		440.0 ± 10.2	8.4 ± 13.3	0
Field-run, New Improved Semesan Bel 1-60	99.6	99	0		434.4 ± 9.5	2.8 ± 12.8	0
Field-run, Sanoseed 1-60	100	100	0		439.6 ± 8.0	8.0 ± 11.7	0
Field-run, yellow mercuric oxide 1-100	100	98	0		430.4 ± 9.0	-1.2 ± 12.4	0
Field-run, corrosive sublimate 1-1200	100	99	0		429.2 ± 10.6	-2.4 ± 13.6	0
Field-run, corrosive sublimate 1-500 and ammonium iodide 1-400	99.2	99	0		431.2 ± 9.2	-0.4 ± 12.6	0
Field-run, corrosive sublimate 1-1000	100	99	0		431.2 ± 8.9	-0.4 ± 12.4	0
Field-run, corrosive sublimate 1-1000 treated 9/22/30	100	100	0	4	428.4 ± 7.2	-3.2 ± 11.2	0
Clean, check, not treated	98.8	99	4	0	434.4 ± 5.9	2.8 ± 10.4	0
Clean, corrosive sublimate 1-1000							

1931 EXPERIMENTS

The results of the experiments conducted in 1931 are recorded in tables 6, 7, and 8. The seed tubers were given the corrosive sublimate and formaldehyde treatments, as in 1930; New Improved Semesan Bel, which was the same as Dubay No. 664 used in 1929; Sanoseed; yellow mercuric oxide 1-100 as a dip as used by Blodgett (2); a solution of corrosive sublimate 1-1200 and potassium iodide 1-400 used as a dip; and corrosive sublimate 1-500 and ammonium carbonate 1-40 used for 3 minutes. The acid corrosive sublimate was used both as a dip and as a 3-minute treatment. The standard corrosive sublimate was used as a fall treatment as well as a spring treatment. The land on which the rhizoctonia-infected Irish Cobblers were they were placed in storage in the fall rather than following removal from storage in the spring, as is the usual practice. White (9) reported good results with fall treatment in Kansas.

Most of the tubers treated in 1931 had sprouts ranging from $\frac{1}{8}$ inch to $\frac{3}{8}$ inch in length. This sprouted condition of the tubers probably was responsible for the injury that resulted in 1931 from some of the treatments. Most of the treatments caused some burning of the sprouts, and the hot formaldehyde seemed to be especially severe in this respect.

In the experiments of 1931 the seed was planted from 5 to 7 days after treatment. The land on which the rhizoctonia-infected Irish Cobblers were planted appeared uniform, but the variation in the yields from the different plots indicated that it was very uneven with respect to productivity.

The control of rhizoctonia, as measured by the infection on the tubers that were produced, was not so good as that obtained in other years. Clean seed tubers included in the experiment on rhizoctonia-infected tubers (Table 6) produced a crop with 16 per cent of the tubers infected, while clean seed tubers in an adjoining experiment (Table 7) in which only clean tubers were used produced a crop with only 1 per cent of the tubers infected. These data suggest that there may have been some spread of rhizoctonia from the diseased to the healthy plots in the experiment recorded in table 6. All of the treatments except the short-time one in cold formaldehyde gave yield increases from the rhizoctonia-infected seed tubers, but the increases apparently were significant only where the regular cold formaldehyde and the corrosive sublimate-potassium iodide treatments were used.

Seed treatment of the clean Irish Cobblers or of the field-run Green Mountains did not result in any significant increases in yield.

In the experiment on clean Irish Cobblers a treated and a nontreated lot of clean approximately $1\frac{1}{2}$ -ounce whole tubers were used as a possible check against seed-piece decay. The amount of seed-piece decay recorded in the experiments of 1931 was negligible, so no advantage for the whole tubers was noted in this regard. The yield of the nontreated whole tubers, how-

TABLE 9.—Seed-potato treatment results—Irish Cobblers 1932

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over diseased nontreated check	Approx- imate odds
Diseased, check, not treated	98.8	89	35	37	335.5 ± 9.2
Diseased, corrosive sublimate 1-1000 1½ hrs.	99.6	92	0	4	355.4 ± 3.2	19.9 ± 9.7	5: 1
Diseased, corrosive sublimate 1-500 and .56% glacial acetic acid 3 min.	100	95	8	16	375.4 ± 5.2	39.9 ± 10.6	95: 1
Diseased, corrosive sublimate 1-500 and 1% commercial hydrochloric acid 3 min.	100	93	8	8	355.4 ± 5.1	19.9 ± 10.5	4: 1
Diseased, corrosive sublimate 1-500 and 1% commercial hydrochloric acid dip	100	94	4	14	352.2 ± 6.2	16.7 ± 11.1	2: 1
Diseased, formaldehyde 1-120, 52° C. 3 min., covered 1 hr.	100	94	10	11	352.2 ± 8.4	16.7 ± 12.5	2: 1
Diseased, New Improved Semesan Bel 1-60 dip	99.2	97	10	16	353.0 ± 5.7	17.5 ± 10.8	3: 1
Diseased, Sanoseed 1-60 dip	100	95	8	12	358.6 ± 5.3	23.1 ± 10.6	6: 1
Diseased, yellow mercuric oxide 1-100 dip	98.8	78	0	3	310.3 ± 8.0	-25.2 ± 12.2	5: 1
Diseased, corrosive sublimate 1-1200 and potassium iodide 1-400 dip	100	95	6	6	348.6 ± 4.2	13.1 ± 10.1	2: 1
Diseased, corrosive sublimate 1-500 and ammonium carbonate 1-20 3 min.	100	93	8	16	334.7 ± 8.4	-0.8 ± 12.5	0
Diseased, corrosive sublimate 1-1000 treated 9/22/31	99.6	93	4	8	349.4 ± 3.9	13.9 ± 10.0	0
Diseased, corrosive sublimate 1-500 and 1% commercial hydrochloric acid 3 min.	99.6	87	2	5	351.0 ± 5.2	15.5 ± 10.6	2: 1
Clean, check, not treated	99.6	98	0	3	367.4 ± 4.1	31.9 ± 10.1	31: 1
Clean, corrosive sublimate 1-1000 1½ hrs.	100	95	2	4	363.0 ± 6.1	27.5 ± 11.0	10: 1

TABLE 10.—Seed-potato treatment results—clean Irish Cobblers 1932

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Bushels	Increase over non- treated check Approximate odds
Check, not treated					328.7 ± 5.9
Corrosive sublimate 1-1000	99.6	97	0	2			
1½ hrs.	99.6	96	0	7	308.3 ± 11.6	-20.4 ± 13.0	3:1
Corrosive sublimate 1-500 and .56% glacial acetic acid							
3 min.	100	97	4		321.1 ± 6.0	-7.6 ± 8.4	0
Corrosive sublimate 1-500 and 1% commercial hydrochloric acid							
3 min.	100	94	0		302.8 ± 5.4	-25.9 ± 8.0	31:1
Corrosive sublimate 1-500 and 1% commercial hydrochloric acid							
dip	98.8	98	0		325.5 ± 3.1	-3.2 ± 6.7	0
Formaldehyde 1-120, 52° C. 3 min., covered 1 hr.	99.6	97	0		323.9 ± 5.3	-4.8 ± 7.9	0
New Improved Semesan Bel 1-60	99.6	96	0		322.3 ± 5.7	-6.4 ± 8.2	0
dip	100	96	4		325.5 ± 4.5	-3.2 ± 7.4	0
Sanoseed 1-60	99.2	88	0		302.8 ± 5.7	-25.9 ± 8.2	31:1
Yellow mercuric oxide 1-100							
Corrosive sublimate 1-1200 and potassium iodide 1-400	99.6	97	0		312.7 ± 3.2	-16.0 ± 6.7	8:1
Corrosive sublimate 1-500 and ammonium carbonate 1-20	100	96	0		321.5 ± 6.0	-7.2 ± 8.4	0
1½-oz. whole tubers, check, not treated	100	99	0		343.5 ± 5.4	14.8 ± 8.0	4:1
1½-oz. whole tubers, corrosive sublimate 1-1000	100	98	0		329.1 ± 7.3	0.4 ± 9.4	0

TABLE 11.—Seed-potato treatment results—Green Mountains 1932

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over field-run nontreated check	
						Bushels	Approxi- mate odds
Field-run, check, not treated	100	99	19	6	386.5 ± 7.9
Field-run, corrosive sublimate 1-1000	99.6	98	6	2	380.2 ± 7.1	-6.3 ± 10.6	0
Field-run, corrosive sublimate 1-500 and .56% glacial acetic acid	100	100	0		387.3 ± 5.9	0.8 ± 9.9	0
Field-run, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	100	100	0		379.8 ± 4.9	-6.7 ± 9.3	0
Field-run, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	100	100	2		375.4 ± 4.6	-11.1 ± 9.1	1:1
Field-run, formaldehyde 1-120, 52° C.							
Field-run, New Improved Semesan Bel 1-60	99.6	100	4		397.3 ± 6.6	10.8 ± 10.3	1:1
Field-run, Sanoseed 1-60	99.6	100	8		379.8 ± 3.1	-6.7 ± 8.5	0
Field-run, yellow mercuric oxide 1-100	100	100	0		392.9 ± 6.4	6.4 ± 10.2	0
Field-run, corrosive sublimate 1-1200	100	99	2		382.5 ± 4.5	-4.0 ± 9.1	0
Field-run, corrosive sublimate 1-500 and and potassium iodide 1-400	100	100	0		407.3 ± 4.4	20.8 ± 9.0	7:1
Field-run, corrosive sublimate 1-500 and ammonium carbonate 1-20	100	100	0		401.7 ± 5.9	15.2 ± 9.9	2:1
Field-run, corrosive sublimate 1-1000 treated 9/22/31	100	100	0		406.1 ± 5.9	19.6 ± 9.9	5:1
Field-run, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	100	99	2		390.1 ± 6.2	3.6 ± 10.0	0
Clean, check, not treated	99.6	100	4	1	401.3 ± 4.4	14.8 ± 9.0	3:1
Clean, corrosive sublimate 1-1000	99.6	100	12	1	399.3 ± 7.4	12.8 ± 10.8	1:1

ever, was 35.5 bushels per acre greater than that from the nontreated cut tubers, but the odds were only approximately 19 to 1.

1932 EXPERIMENTS

The experiments in 1932 were much the same as those of 1931. Tables 9, 10, and 11 give the results. In the experiment where infected tubers were used the injury due to rhizoctonia was not so great as that occurring in previous years. In the nontreated check only 35 per cent of the plants showed stem lesions and only 37 per cent of the tubers that were produced showed rhizoctonia sclerotia. Most of the treatments used reduced these percentages to relatively low figures. The seed pieces were covered to a depth of about $4\frac{1}{2}$ inches as compared to about 6 inches in previous years.

The corrosive sublimate with the glacial acetic acid was the only treatment that caused a significant increase in yield of the rhizoctonia-infected Irish Cobblers but its control of rhizoctonia was not good, as indicated by the fact that 16 per cent of the tubers harvested bore rhizoctonia sclerotia.

All of the treated lots of clean Irish Cobblers yielded less than the nontreated check but the differences apparently were significant only where the acid corrosive sublimate and the yellow mercuric oxide treatments were used.

The treatments did not cause any significant difference in the yield of the field-run Green Mountains.

1933 EXPERIMENTS

The results of experiments of 1933 are given in tables 12 and 13. The treatments used in 1933 on rhizoctonia-infected Irish Cobblers included the standard corrosive sublimate, the acid corrosive sublimate and various modifications of it, and 4 treatments using corrosive sublimate and potassium iodide. Only 3 treatments, the standard corrosive sublimate, the acid corrosive sublimate, and the corrosive sublimate and potassium iodide, were used on clean Irish Cobblers. An additional check of clean $1\frac{1}{2}$ -ounce whole tubers was included in this experiment. No Green Mountains were treated in 1933.

The chemically pure hydrochloric acid was compared with the commercial hydrochloric in the acidulated corrosive sublimate treatment to determine whether or not the pitting caused by this treatment might be due in part to impurities in the acid. Both treatments gave similar results, so that on the basis of this experiment one grade of acid cannot be considered better than the other.

Of the modifications of the acidulated corrosive sublimate treatment, none seemed to be superior to the original. It is of interest that the dip treatments in which the strength was slightly increased gave good results; but these combinations should be subjected to further trial, since rhizoctonia was not very severe in this experiment.

TABLE 12.—Seed-potato treatment results—Irish Cobblers 1933

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over diseased nontreated check	Approx- imate odds
Diseased, check, not treated	96.5	87	42	15	239.7 ± 6.9	11.2 ± 9.1	1:1
Diseased, corrosive sublimate 1-1000	99.6	91	0	0	250.9 ± 5.9		
Diseased, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	99.6	90	8	1	251.7 ± 6.1	12.0 ± 9.2	2:1
Diseased, corrosive sublimate 1-500 and 1% C.P. hydrochloric acid	96.9	88	8	1	247.7 ± 4.0	8.0 ± 8.0	1:1
Diseased, corrosive sublimate 1-1000 and 1% commercial hydrochloric acid	95.4	86	6	3	253.3 ± 5.6	13.6 ± 8.9	2:1
Diseased, corrosive sublimate 1-400 and 1% commercial hydrochloric acid	97.3	92	0	3	251.7 ± 3.7	12.0 ± 7.8	2:1
Diseased, corrosive sublimate 1-500 and 2% commercial hydrochloric acid	98.1	90	4	3	254.9 ± 3.7	15.2 ± 7.8	4:1
Diseased, corrosive sublimate 1-550 and 1% commercial hydrochloric acid	98.8	90	4	1	257.3 ± 3.4	17.6 ± 7.7	7:1
Diseased, corrosive sublimate 1-1200 and potassium iodide 1-400	100	94	10	7	254.1 ± 5.7	14.4 ± 8.9	3:1
Diseased, corrosive sublimate 1-1000 and potassium iodide 3-1000	100	95	15	4	265.7 ± 4.7	26.0 ± 8.3	26:1
Diseased, corrosive sublimate 1-1200 and potassium iodide 1-400, 5th time used	100	94	12	3	249.3 ± 4.1	9.6 ± 8.0	1:1
Diseased, corrosive sublimate 1-1200 and potassium iodide 1-400, 10th time used	98.8	95	4	2	254.1 ± 3.3	14.4 ± 7.6	4:1
Clean, check, not treated	100	97	0	0	250.5 ± 4.5	10.8 ± 8.2	2:1
Clean, corrosive sublimate 1-500 and 1% commercial hydrochloric acid	99.2	94	0	1	258.1 ± 5.1	18.4 ± 8.6	5:1

TABLE 13.—Seed-potato treatment results—clean Irish Cobblers 1933

Treatment	Percent- age of stand	Relative vigor	Percent- age with stem lesions	Percent- age with tuber infection	Marketable tubers		
					Yield per acre in bushels	Increase over non- treated check	Approximate odds
Check, not treated	99.6	96	2	0	245.3 ± 6.2
Corrosive sublimate 1-1000 1½ hrs.	98.5	92	0	1	237.7 ± 7.1	-7.6 ± 9.4	0
Corrosive sublimate 1-500 and 1% commercial hydrochloric acid 3 min.	96.5	86	0		239.3 ± 5.8	-6.0 ± 8.5	0
Corrosive sublimate 1-1200 and potassium iodide 1-400 dip	98.8	96	0		241.3 ± 8.4	-4.0 ± 10.4	0
1½-oz. whole tubers, check, not treated	100	98	0		268.5 ± 8.8	23.2 ± 10.8	5:1

The relative vigor of the plants from tubers treated with corrosive sublimate in combination with potassium iodide was slightly higher than that of plants from tubers treated with the standard corrosive sublimate or with the acid corrosive sublimate mixtures.

In order to get information on the rate of loss of strength of the corrosive sublimate and potassium iodide treatment 1 lot was planted that was the first one dipped in a fresh solution, another one planted was the fifth, and another the tenth, starting with a little more solution than that necessary to cover a given lot of tubers. The last lot treated showed the best control, so there was no indication of loss of strength in these tests.

The yields in 1933 were low because of drought. The percentage of tubers bearing rhizoctonia sclerotia at harvest time was low also.

All of the treatments of the rhizoctonia-infected tubers gave increases in yield, but the increases were not significant, except in the case where a solution of corrosive sublimate 1-1000 and potassium iodide 3-1000 was used.

All of the treated lots of the clean Irish Cobblers yielded less than the nontreated control, but the differences were not significant.

DISCUSSION AND CONCLUSION

Considerable variation from year to year was experienced in these seed-potato treatment experiments. Such variation is to be expected since it is known that variations in soil moisture and temperature have a marked influence on the organism as well as on the host. The fact that the seed pieces were covered to a depth of about 6 inches in 1929, 1930, and 1931 and to a depth of about 4½ inches in 1932 and 1933 may have caused some of the differences recorded, since it is known that deep covering favors rhizoctonia. The differences recorded with the cold formaldehyde 1-120 used for 3 minutes are difficult to explain. This treatment was not expected to give good control, but was included for comparison with the 3-minute treatment with corrosive sublimate. In 1929 it gave satisfactory control of rhizoctonia but in 1931 and 1932 it was almost a complete failure in this regard. The only differences in the treatment in 1929, as compared with this treatment of the other years, were that the tubers were not given a 1-hour covering after treatment and that the temperature of the solution was slightly higher.

Seed-piece decay was not a factor in these experiments from 1929 to 1933. It is probable that handling the potatoes in bushel crates, that allowed the passage of air after treatment and cutting, contributed to these good results. The amount of benefit that might result from the treatment of seed, that must be held after cutting for long periods under less favorable conditions, has not been determined. It is known that seed-piece decay sometimes is an important factor in potato production and that in such instances seed tuber treatment is beneficial (7).

Notes were taken on the severity of stem lesions and on the degree of tuber infection. Since these figures, in general, corresponded closely with those for the percentage of stem lesions and the percentage of tubers infected, respectively, they were not included in the tables. In other words, where the percentage of stem lesions was high, the average lesion was more severe than where the percentage of stem lesions was low; likewise, where the percentage of infected tubers was high, the area covered by sclerotia usually was high.

The standard corrosive sublimate treatment controlled rhizoctonia satisfactorily without seriously injuring the tubers, but it has the disadvantage of being a long-time soak.

The acid corrosive sublimate treatment (corrosive sublimate 1-500 and 1 per cent commercial hydrochloric acid) used as a 3-minute soak, compared favorably with the standard corrosive sublimate treatment in control of rhizoctonia, but in nearly all instances it caused more pitting of the treated surface of the tubers. This treatment, as used by a few Maine growers in 1933, resulted in severe pitting of the eyes of the tubers and loss of stand in some instances. It is known that some of these growers did not dry the tubers properly after treatment. Whether other factors were involved has not been determined. In our experiments we have not experienced this severe pitting of the eyes, but we did get what proved to be a significant decrease in yield on clean Irish Cobblers in one experiment. At least, results indicate that this treatment should be used with caution.

The acid (hydrochloric or acetic) corrosive sublimate treatments were much more effective than corrosive sublimate of the same strength, without the acid, used for the same length of time. Glacial acetic acid first was used as 0.8 per cent with corrosive sublimate 1-500, but when it was compared with the regular acidulated corrosive sublimate treatment, using 1 per cent commercial hydrochloric acid, it was used at 0.56 per cent, since that strength gave about the same acidity, as determined by titrating against an alkali, as 1 per cent commercial hydrochloric acid. The acetic acid treatment as used was not as effective in the control of rhizoctonia to the commercial hydrochloric acid treatment. Also it did not pit the tubers as badly. Under certain conditions a blue mold grew on the tubers treated with the acetic acid mixture. This did not result in any noticeable effect but it was not observed where the hydrochloric acid was used.

The yellow mercuric oxide treatment, as used at 1-100, caused what might be considered a burning of the cut surface of the tubers when they came in contact with the treated surface after the tubers were cut. Also this treatment appeared to have a marked retarding effect on the early growth of the plants in some instances. Its control of rhizoctonia was very good. A more dilute mixture might have given more favorable results. In New York, yellow mercuric oxide was tried at 1-100 as a dip (2) and later was recom-

mended at the rate of 1 pound of yellow mercuric oxide to 15 gallons of water (8).

Laboratory tests indicated that a solution of corrosive sublimate 1-1200 and potassium iodide 1-400 used as a dip treatment (6) was effective in the control of the growth of *rhizoctonia sclerotia*. Field tests reported here, in general, substantiate these results. The high cost of the potassium iodide is a disadvantage of this treatment. It is well known that when potassium iodide is added to a solution of corrosive sublimate a yellow, then red, precipitate of mercuric iodide is formed that in turn is soluble in an excess of potassium iodide. It is this final solution that was used in these experiments, and, in order to get it, the ratio of potassium iodide to corrosive sublimate must be, at least, about 3 to 1.

The organic mercuries, Sanoseed and New Improved Semesan Bel, as used in these experiments, were fairly effective in the control of *rhizoctonia*. They have the advantage of being dip treatments.

Seed potatoes, selected as clean, when planted in a separate experiment, either treated or nontreated, over a period of 5 years, never produced a crop having over 7 per cent of the tubers infected with *rhizoctonia*. In most instances only 1 or 2 per cent of the tubers showed any *sclerotia*, even when washed. These results indicate that, under the conditions of these experiments, a program of selection and treatment would be more desirable than one of treatment alone, especially if the crop is to be used for seed purposes. The inclusion of selection in the control program is made even more desirable by the fact that none of the present treatments give perfect control of *rhizoctonia* without chance of injury to the potatoes.

The effect of crop rotation on *rhizoctonia* has not been determined under Maine conditions. All of these experiments were conducted on land that was in a 3-year rotation of oats, clover, and potatoes.

SUMMARY

The results of seed-potato treatment experiments for the years 1929 to 1933 are recorded.

In general, the plans of the experiments were similar to those of experiments conducted in Maine in 1927 and 1928.

Seed-tuber treatment of *rhizoctonia*-infected Irish Cobblers usually increased the stand (the percentage of plants that emerged), increased the average vigor of the plants, reduced the injury from stem lesions, reduced the percentage of infected tubers, and increased the yield.

Seed-tuber treatment of clean Irish Cobblers and field-run Green Mountains did not increase the yield. Tuber treatment of this type of potatoes might be regarded as an insurance against *rhizoctonia* that might develop over a period of years if the strain of potatoes is planted continuously with-

out selection and as an insurance against seed-piece decay, which occurs under some conditions.

Seed-piece decay was not a factor in these experiments.

The standard corrosive sublimate, $1\frac{1}{2}$ -hour soak, treatment was one of the most effective treatments used from the standpoint of rhizoctonia control.

The acidulated corrosive sublimate (corrosive sublimate 1-500 with 1 per cent commercial hydrochloric acid) 3-minute treatment, was satisfactory regarding rhizoctonia control but it seemed to be somewhat more dangerous from the standpoint of injury than the standard corrosive sublimate treatment. Special care should be taken that tubers are dried after treatment.

Yellow mercuric oxide 1-100, used as a dip, gave good control of rhizoctonia but was injurious in some instances.

A solution of corrosive sublimate 1-1200 and potassium iodide 1-400, used as a dip treatment, gave promising results in these experiments.

The organic mercury dips, Sanoseed and New Improved Semesan Bel, proved to be superior to organic mercury dips sold in previous years. As used they were slightly inferior to the standard corrosive sublimate treatment in regard to rhizoctonia control.

The term "dip treatment," as used, indicates that the tubers were dipped 3 times in rapid succession or that they were placed in a solution, agitated slightly, and then removed. In either case thorough wetting resulted.

Clean Irish Cobblers, when planted in a separate experiment, either treated or not, produced a crop bearing very few rhizoctonia sclerotia.

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THE EFFECT OF SEVERAL COLLECTIONS OF *TILLETIA TRITICI* AND *T. LEVIS* ON THE MORPHOLOGY OF SPRING WHEATS¹

O. S. AAMODT, J. H. TORRIE, AND K. TAKAHASHI²

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INTRODUCTION

In the course of bunt studies conducted at the University of Alberta, Edmonton, Canada, striking morphological differences were observed in wheat plants infected with *Tilletia*. The most striking of the effects observed were: the reduction in culm length, elongation of the spike, and the shape of the bunt ball. Since there appeared to exist differences among the wheats grown in the bunt test for the above characters, it was considered desirable to secure more definite information on the influence of different collections of bunt on several varieties for these morphological changes. Other workers have reported morphological changes in wheat resulting from infection by the bunt fungi. The natures of these reports are somewhat conflicting.

MATERIALS AND METHODS

The material was grown at the University of Alberta in 1931. Six varieties of spring wheat: Reward, C.A.N.³ 1509, Little Club, C.A.N. 1620, Pentad, C.A.N. 1633, Hope, C.A.N. 1615, Garnet, C.A.N. 1316 and Kota, C.A.N. 1364, were used as hosts. The seed of the 6 varieties was inoculated separately with chlamydospores from five collections of bunt. The collections of bunt consisted of one of *Tilletia tritici* (Bjerk.) Wint. obtained from Garnet grown at Egremont, Alberta; and 4 of *T. levis* (Kühn), which were obtained from Garnet, Marquis, Reward, and H-44, grown in the experimental plots at the University of Alberta in 1930. These collections have been designated by the numbers 1, 2, 3, 4, and 5, respectively.

The method of inoculation consisted of applying approximately equal amounts of dry chlamydospores to the seed, in an envelope. The envelope containing the mixture of seed and spores was then shaken thoroughly. Precautions were taken to avoid mixing of the inoculum in the preparation of the several samples and in the sowing. Hands and equipment were thoroughly washed in alcohol between handlings of the different lots of

¹ Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada.

² Professor of Genetics and Plant Breeding, Research Assistant, and Graduate Student, respectively.

³ Canadian accession number.

material. The inoculated seed was sown in duplicate at the rate of 100 seeds to a 10-foot row. Noninoculated seed of each variety was sown as a check. At maturity the plants from the duplicate rows were pulled and bulked.

Certain of the inoculated plants showed no infection, others were partially infected, and still others completely so. As far as the material permitted, 20 plants for each of these 3 classes of plants from inoculated seed were selected at random. The material was studied in detail to find the reaction of the several wheat varieties to the different collections of bunt in regard to culm length, spike elongation, bunt-ball shape, and any possible interrelationships between these characters.

The culm length was measured in centimeters from the crown of the plant to the base of the spike for each culm of every plant studied. The spikes were classified into 7 classes for spike elongation. The classes ranged from 1, indicating a shortening of the spike, to 7, indicating considerable elongation. The bunt balls were classified into 5 classes from 1 to 5 on the basis of their length in proportion to width, those in class 1 being round, while those in class 5 were long and narrow. A statistical analysis of the data was made after the completion of the preliminary notes.

CULM LENGTH

Although the effect of bunt on the culm length of the wheat plant has been observed by a large number of workers, very little comprehensive work has been published. Harwood (5), in Michigan, observed a difference in the height of wheat plants infected with *Tilletia levis* and *T. tritici*. He states that the culms of wheat infected with *T. tritici* (low smut) grew only to a height of 1 to 1.5 feet, while those infected with *T. levis* (high smut) were little if any shorter than the culms of noninfected plants. Potter and Coons (8) confirmed Harwood's observations, stating that the culms of wheat plants infected with *T. levis* were from 2 to 4 inches shorter, while those infected with *T. tritici* were 12 inches shorter than the culms of noninfected plants. They also report that plants partially infected with *T. tritici* were taller than completely infected plants. Bressman (2) studied ten wheat varieties, each inoculated separately with 43 different collections of bunt. He concludes that, in general, the culms of plants infected with *T. tritici* are shorter than those infected with *T. levis* and that in both the culms are shorter than those of noninfected plants. However, he states that this difference in culm length is not a constant characteristic of the species.

Other investigators report no difference in the culm length of wheat infected with the 2 species of *Tilletia*. Selby (10), in Ohio, found no difference in the relative degree of stunting of wheat infected with *T. levis* or *T. tritici*. McAlpine (7), in Australia, arrived at similar conclusions.

Rodenhiser (9) demonstrated that both *T. levis* and *T. tritici* may cause significant reductions in the culm length of the wheat plant. He states that whether or not significant differences occur in the relative degree of stunting caused by the 2 species of *Tilletia* depends on the physiologic form with which the plant is infected. He suggests that it is entirely possible that the physiologic forms tested might have reacted differently on other varieties and under different environmental conditions.

A number of workers have studied the effect of one or the other species of *Tilletia* on the culm length of wheat plants. Barrus (1) observed a shortening of the culms of diseased plants, as compared with those of normal plants, when infected with *T. levis*. This, he states, is due to a reduction in length of all the internodes. Woolman and Humphrey (15) report a greater shortening of the culms of *Triticum compactum* as compared with those of *T. vulgare*, when attacked with bunt. Vilkaitis (13) found that *Tilletia tritici* reduces the culm length of an unspecified wheat by as much as 25 per cent. Viennot (12) reports that the reduction of height of Bon Fermier wheat infected with *T. tritici* is due to a decrease in the number of internodes more than to a shortening between the internodes.

EXPERIMENTAL RESULTS

The plants studied were placed in 4 groups as follows: nonbunted plants from the noninoculated seed; nonbunted plants from inoculated seed; and partially bunted and completely bunted plants from inoculated seed. The nonbunted and bunted culms of partially bunted plants were treated separately. The data given in table 1 show the effect of the several collections of bunt on the culm length of the 6 wheat varieties for the different groupings. The data for the differences among and significance for the various groupings are given in table 2. The method employed in calculating the significance of the differences among the various means was Fisher's (4) method of obtaining the significance of differences among the means of small samples that are not paired in any way. The value of P was obtained from Fisher's Table of "t."

An examination of the data in tables 1 and 2 reveals many interesting observations. The difference in culm length of plants grown from non-inoculated seed and nonbunted plants grown from inoculated seed, is significant in 11 cases out of 17. The differences are significant for Reward, when inoculated with collections 1 and 2, but not with collections 3 and 5; for Little Club, with 1, 2, and 3, but not with 4; for Pentad, with 2, 3, and 4, but not with 1; for Hope, with 1 but not with 2; and Kota, with 1 and 2 but not with 5. These data indicate that almost all plants become infected when inoculated, and that resistance as ordinarily used is the suppression of the fungus sufficiently so that bunted grain is not produced, rather than

the ability to inhibit infection, though the latter may also be a form of resistance. Infection is evidenced by the shortened culms of nonbunted plants grown from inoculated seed. A detailed histological study would be necessary to verify this evidence.

A comparison of the culm length between the nonbunted culms of nonbunted and partially bunted inoculated plants shows that in 6 cases out of 19 the differences are significant. In 2 of the 6 cases where the differences were significant, the nonbunted culms of partially bunted plants were the longest. No satisfactory reason can be offered for the occurrence of these significant differences, especially when, in certain cases, the culms of nonbunted inoculated plants are the longer; while in other cases the nonbunted culms of partially bunted plants are the longer. The lack of significant differences in most of the cases and the fact that, where the differences are significant, they are not uniformly in one direction, lends support to the hypothesis that nearly all plants become infected when inoculated.

Several comparisons were used in studying the differences in culm length between nonbunted and bunted culms, namely, noninoculated and completely bunted plants; nonbunted inoculated and bunted culms of partially bunted plants; nonbunted inoculated and completely bunted plants, nonbunted culms of partially bunted plants and the culms of completely bunted plants; and nonbunted and bunted culms of partially bunted plants. In every comparison the difference was significant, save with Little Club inoculated with collections 1, 2, 3, and 4 for the nonbunted and bunted culms of partially bunted plants. The culm length of nonbunted inoculated plants for Little Club with all collections, were longer than the nonbunted culms of partially infected plants, and were significant in 3 out of 4 cases. Also, it is interesting to note that, for Little Club, with all collections, the bunted culms of partially infected plants were longer than the bunted culms of completely infected plants, and that in 3 out of the 4 cases the difference was significant. No definite reason can be given for this apparent peculiar behavior of Little Club.

A comparison of the bunted culms of partially bunted plants and the culms of completely bunted plants shows that in all cases the bunted culms of partially bunted plants are the taller. However, the differences are only significant in 4 out of 15 comparisons, namely, collections 1 on Reward and 1, 2 and 3 on Little Club.

The data clearly indicate that infection of a wheat plant with either *Tilletia levis* or *T. tritici* may cause a significant reduction in culm length. In the degree of stunting the various collections appear to act somewhat differently on the several wheat varieties. This is shown by the reaction of Reward, Little Club and Pentad in culm length when infected with collections 1, 2 and 3. A much greater reduction resulted in the mean culm

TABLE 1.—Effect of several collections of bunt on the culm length, in centimeters, of six wheat varieties

Variety	Bunt collection				
	1	2	3	4	5
REWARD					
<i>Seed not inoculated</i>	96.6 ± 1.39 ^a
Plants nonbunted
<i>Seed inoculated</i>					
Plants nonbunted	90.8 ± 1.39	85.6 ± 1.45	95.5 ± 1.29	95.8 ± 1.10
Plants partially bunted:					
Nonbunted culms	93.3 ± 1.39	91.1 ± 2.15	95.6 ± 1.60	97.6 ± 2.45
Bunted culms	82.7 ± 0.98	81.1 ± 1.56	88.2 ± 1.32	88.0 ± 1.41
Plants completely bunted	77.9 ± 1.16	78.5 ± 1.13	86.6 ± 1.20	87.7 ± 1.10
LITTLE CLUB					
<i>Seed not inoculated</i>	101.0 ± 1.54
Plants nonbunted
<i>Seed inoculated</i>					
Plants nonbunted	86.1 ± 1.05	94.9 ± 1.10	91.7 ± 1.29	100.0 ± 1.08
Plants partially bunted:					
Nonbunted culms	84.7 ± 1.72	90.6 ± 1.54	87.5 ± 0.95	93.1 ± 1.70
Bunted culms	80.5 ± 1.59	86.6 ± 1.69	86.1 ± 1.59	88.5 ± 2.06
Plants completely bunted	74.0 ± 1.19	81.5 ± 1.29	81.6 ± 1.22	86.4 ± 1.42
PENTAD					
<i>Seed not inoculated</i>	110.3 ± 1.25
Plants nonbunted
<i>Seed inoculated</i>					
Plants nonbunted	110.9 ± 1.38	100.2 ± 1.51	105.6 ± 1.20	103.3 ± 1.30
Plants partially bunted:					
Nonbunted culms	105.2 ± 1.75	100.7 ± 2.19	104.1 ± 2.06	103.8 ± 1.93
Bunted culms	84.6 ± 2.36	79.3 ± 2.74	86.1 ± 2.42	84.1 ± 1.99
Plants completely bunted	77.0 ± 2.82	80.2 ± 2.34

TABLE 1.—(Continued)

Variety	Bunt collection				
	1	2	3	4	5
HOPE					
Seed not inoculated
Plants nonbunted	96.6 ± 1.59
Seed inoculated					
Plants nonbunted	92.2 ± 1.54	96.9 ± 1.17
Plants partially bunted:					
Nonbunted culms	93.3 ± 1.10	93.9 ± 2.37
Bunted culms	84.0 ± 1.19	84.7 ± 3.38
Plants completely bunted
KOTA					
Seed not inoculated	111.5 ± 1.20
Plants nonbunted
Seed inoculated					
Plants nonbunted	102.6 ± 2.30	95.2 ± 1.11	112.1 ± 1.54
Plants partially bunted:					
Nonbunted culms	101.3 ± 1.72	92.5 ± 2.22	117.3 ± 2.02
Bunted culms	89.3 ± 1.57	85.4 ± 1.48	101.9 ± 1.44
Plants completely bunted	87.5 ± 1.17	82.7 ± 1.50	99.8 ± 1.48
GARNET					
Seed not inoculated
Plants nonbunted
Seed inoculated					
Plants nonbunted	99.7 ± 0.83	97.2 0.62	98.4 ± 0.96
Plants partially bunted:					
Nonbunted culms	97.7 ± 1.38	96.7 ± 1.41	95.7 ± 1.41
Bunted culms	87.7 ± 1.62	87.2 ± 1.78	91.9 ± 1.14
Plants completely bunted	87.0 ± 2.37

a Mean with standard error.

length for Reward infected with collections 1 and 2 than when infected with collection 3; Little Club, infected with collection 1 than with collections 2 and 3; and Pentad, infected with collection 2 than with collections 1 and 3.

No consistent evidence was obtained to lend support to the opinion that the culms of wheat plants infected with *Tilletia tritici* are shorter than those infected with *T. levis*, which is in agreement with the results reported by Selby (10), McAlpine (7), and Rodenhiser (9); but not with those of Harwood (5) and Potter and Coons (8). The evidence presented lends support to the statement of Rodenhiser (9) to the effect that, whether or not there is a significant difference in the relative degree of stunting caused by the two species of *Tilletia*, depends upon the physiologic form of *Tilletia* with which the plants are infected.

TABLE 2.—Differences in average culm length in centimeters between various groupings of six wheat varieties inoculated separately with several collections of bunt

Variety	Bunt collection				
	1	2	3	4	5
REWARD					
1. Nonbunted plants, seed not inoculated and inoculated	5.8 ± 1.9 ^a	10.6 ± 1.9 ^a	0.7 ± 1.9	0.4 ± 1.8
2. Nonbunted culms of nonbunted and partially bunted inoculated plants	2.5 ± 1.9	5.5 ± 2.2 ^a	0.1 ± 1.8	1.8 ± 2.2
3. Noninoculated plants and completely bunted plants	18.7 ± 1.8 ^a	17.7 ± 1.8 ^a	9.6 ± 1.8 ^a	8.5 ± 1.8 ^a
4. Nonbunted plants and bunted culms, partially bunted inoculated plants	8.1 ± 1.6 ^a	4.5 ± 2.1 ^a	7.3 ± 1.9 ^a	7.8 ± 1.8 ^a
5. Nonbunted and completely bunted inoculated plants	12.9 ± 1.8 ^a	6.9 ± 1.8 ^a	8.8 ± 1.8 ^a	8.1 ± 1.6 ^a
6. Nonbunted and bunted culms of partially bunted plants	10.6 ± 1.8 ^a	9.0 ± 2.7 ^a	7.4 ± 2.1 ^a	9.6 ± 2.8 ^a
7. Nonbunted culms partially bunted plants and completely bunted plants	15.3 ± 1.8 ^a	12.6 ± 2.4 ^a	9.0 ± 2.1 ^a	9.9 ± 2.7 ^a
8. Bunted culms of partially and completely bunted plants	4.9 ± 1.5 ^a	2.6 ± 1.9	1.6 ± 1.8	0.3 ± 1.9

TABLE 2.—(Continued)

Variety	Bunt collection				
	1	2	3	4	5
ITTLE CLUB^b					
1	14.9 ± 1.9 ^a	5.1 ± 1.9 ^a	9.3 ± 2.1 ^a	1.0 ± 1.9 ^a
2	1.9 ± 2.2	4.3 ± 1.9 ^a	4.2 ± 1.6 ^a	6.9 ± 2.1 ^a
3	27.0 ± 1.9 ^a	19.5 ± 1.9 ^a	19.4 ± 1.9 ^a	14.6 ± 2.1 ^a
4	5.6 ± 1.9 ^a	8.3 ± 2.1 ^a	5.6 ± 2.1 ^a	11.5 ± 2.4 ^a
5	12.1 ± 1.6 ^a	13.4 ± 1.6 ^a	10.1 ± 1.8 ^a	13.6 ± 1.8 ^a
6	4.2 ± 2.4	4.0 ± 2.4	1.4 ± 1.9	4.7 ± 2.7
7	10.8 ± 2.1 ^a	9.1 ± 1.9 ^a	5.9 ± 1.5 ^a	6.7 ± 2.2 ^a
8	6.6 ± 2.1 ^a	5.1 ± 2.1 ^a	4.5 ± 1.9 ^a	2.1 ± 2.5 ^a
PENTAD					
1	0.6 ± 1.9	10.1 ± 1.9 ^a	4.7 ± 1.8 ^a	7.0 ± 1.8 ^a
2	5.7 ± 2.2 ^a	0.5 ± 2.7	1.5 ± 2.4	0.5 ± 2.4
3	33.3 ± 3.1 ^a	30.1 ± 2.7 ^a
4	26.3 ± 2.7 ^a	20.9 ± 3.1 ^a	19.5 ± 2.7 ^a	19.2 ± 2.4 ^a
5	23.2 ± 3.2 ^a	23.1 ± 2.7 ^a
6	20.6 ± 3.0 ^a	21.4 ± 3.5 ^a	18.0 ± 2.9 ^a	19.7 ± 2.9 ^a
7	23.7 ± 3.5 ^a	23.6 ± 3.0 ^a
8	2.3 ± 3.8	3.9 ± 3.1
HOPE					
1	4.4 ± 2.1 ^a	0.3 ± 2.1
2	1.1 ± 1.9	3.0 ± 2.7
3
4	9.3 ± 1.6 ^a	12.2 ± 3.5 ^a
5
6	8.2 ± 1.9 ^a	9.2 ± 4.1 ^a
7
8
KOTA					
1	8.9 ± 2.7 ^a	16.3 ± 1.6 ^a	0.6 ± 1.9
2	1.3 ± 2.8	2.7 ± 2.5	5.2 ± 2.1 ^a
3	24.0 ± 1.6 ^a	28.8 ± 1.9 ^a	11.7 ± 1.9 ^a
4	13.3 ± 2.8 ^a	9.8 ± 1.8 ^a	10.2 ± 2.1 ^a
5	15.1 ± 2.5 ^a	12.5 ± 1.8 ^a	12.3 ± 1.5 ^a
6	12.0 ± 2.4 ^a	7.1 ± 2.7 ^a	15.4 ± 2.5 ^a
7	13.8 ± 2.1 ^a	9.8 ± 2.2 ^a	17.5 ± 2.5 ^a
8	1.8 ± 1.9	2.7 ± 2.1	2.1 ± 2.1
GARNET					
1
2	2.0 ± 1.6	0.5 ± 1.5	2.7 ± 1.9
3
4	12.0 ± 1.8 ^a	9.7 ± 1.4 ^a	6.5 ± 1.5 ^a
5	10.0 ± 1.9 ^a	11.4 ± 2.5 ^a
6	10.0 ± 2.1 ^a	8.2 ± 1.9 ^a	3.8 ± 1.8 ^a
7	9.5 ± 2.2 ^a	8.7 ± 2.7 ^a
8	2.3 ± 2.2	4.9 ± 2.7

^a P value less than 0.05.^b Numbers refer to enumerated designations in column 1 of table 2.

SPIKE ELONGATION

Heald and Woolman (6) observed that the heads of club varieties of wheat affected with bunt are more slender in form than are normal heads; while those of the Fife or Bluestem varieties do not show such a noticeable change. In the latter, the infected heads have a more loose or open appearance than normal heads, due to a divergence of the glumes caused by an enlargement of the smutted kernels. Barrus (1) observed that the heads infected with *Tilletia levis* of a variety of *Triticum vulgare*, similar to Dawson's Golden Chaff, were shorter and slimmer than those from normal plants. Potter and Coons (8), 1918, state that the heads of wheat plants infected with *Tilletia tritici* are enlarged and present a more crowded appearance than do normal heads; while those infected with *T. levis* are more slender and open in appearance. Woolman and Humphrey (15) report that bunted heads of *Triticum compactum* wheats are much elongated, approaching those of the *T. vulgare* group in form. However, there are frequent exceptions, particularly among the spring-sown clubs. Partially infected heads, they found, were more normal in size and shape than completely infected heads.

Weston (14) reports that winter-sown American Club wheat, infected with bunt, produces much-elongated heads, which resemble those of the *Triticum vulgare* group. He states that such marked elongation was not noted in the species of wheat under observation; viz., *T. sphaerococcum* Perc., *T. spelta* L., *T. polonicum* L., *T. durum* Desf., *T. dicoccum* Schr., *T. persicum* Vav. and *T. turgidum* L. He cites the works of Appel and Edler, both of whom report an elongation of the head in spring-sown club wheats, when affected with bunt. Vilkaitis (13) found a shortening of the head of an unspecified wheat by 15 per cent when infected with *T. tritici*. Rodenhiser (9) observed differences in host reaction for the general shape of heads affected with bunt, but states that these differences were not constant for a single species of *Tilletia*. Bressman (2) obtained an elongation of the spike in the club varieties Albit and Hybrid 128, when affected with bunt. Smith (11) studied the reaction of Martin wheat to different physiologic forms of *T. tritici*. He found that heads on plants infected with physiologic form T₂ were more lax and narrower than those infected with form T₃.

EXPERIMENTAL RESULTS

The infected spikes were classified into seven classes for the degree of spike elongation, namely: 1 indicating a slight shortening, 2 indicating no elongation, and 3 to 7 indicating elongation of various degrees. The data in table 3 give the effect of bunt infection on the spike length for both the partially and completely infected plants of the six wheat varieties, inoculated separately, with the different bunt collections. An analysis of these data shows that spike elongation, resulting from bunt infection, occurred

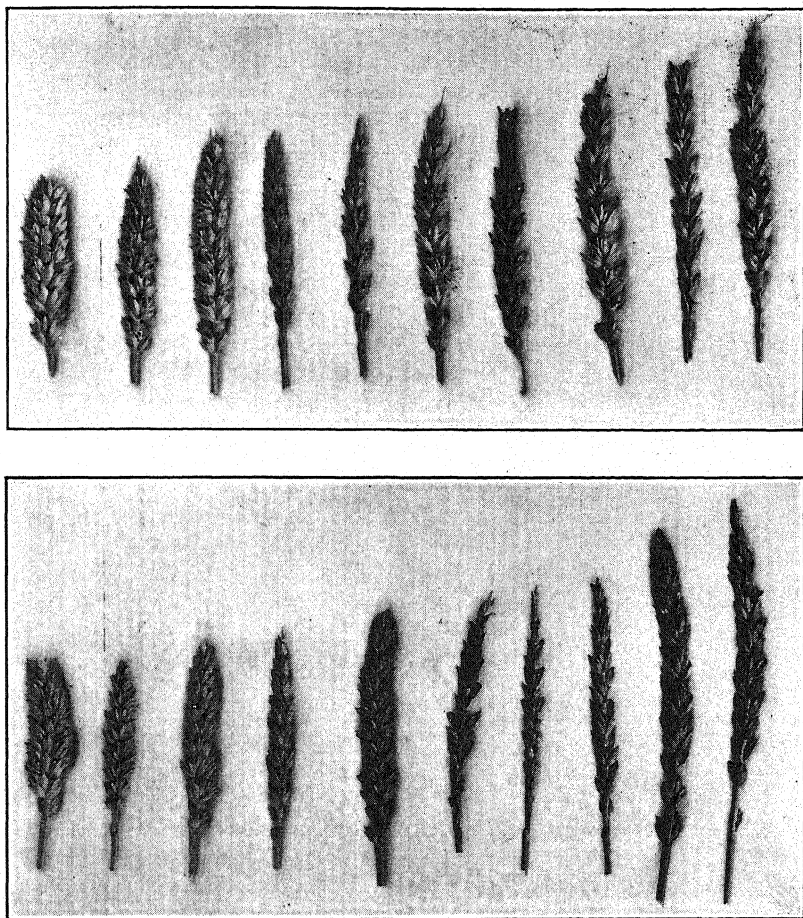


FIG. 1. Extent and great degree of variability in spike elongation, found in infected heads of Little Club for collection 1 of *Tilletia tritici* (upper) and collection 2 of *T. levis*, both of which were obtained from Garnet (lower).

only in the more susceptible varieties of Reward, Little Club and Kota; while in the case of the more resistant varieties of Pentad, Hope and Garnet, little or no spike elongation was found. The susceptible varieties reacted somewhat differently to the several collections of bunt fungi used. Little Club showed considerable elongation of the spike for all collections. In figure 1 are shown heads of Little Club wheat both normal and infected with collection 1 (*T. tritici*) and collection 2 (*T. levis*), both obtained from Garnet. This figure illustrates both the extent of, and the great degree of variability in, the spike elongation found among the heads of Little Club wheat infected separately with both species of *Tilletia*. Little or no elonga-

TABLE 3.—*The effect of the several collections of bunt on the length of infected heads of partially and completely bunted plants of six wheat varieties*

Variety and classes for spike elongation	Bunt collection and number of heads									
	1		2		3		4		5	
	P ^a	C ^b	P	C	P	C	P	C	P	C
<i>Reward</i>										
1 ^c			1	1					2	
2	50	57	25	24	23	31			43	38
3	3	9	17	18	18	22			6	9
4			7	6	4	8				
5				2	3	1				
6				1						
7										
<i>Little Club</i>										
1				2	1	2				
2	17	31	13	20	9	24	6	11		
3	22	21	15	18	23	35	13	18		
4	10	25	5	7	16	17	14	26		
5	8	3	3	2	2	5	6	7		
6	2		1	2		1		1		
7			2							
<i>Pentad</i>										
1										
2	33		33	18	23	6	33	20		
3			3	2	9		8	3		
4										
5										
6										
7										
<i>Hope</i>										
1										
2	14		20							
3										
4										
5										
6										
7										
<i>Kota</i>										
1		1	2	2					3	3
2	39	56	22	34					34	46
3	5	3	14	17					7	9
4	1		4	4					4	2
5			1	1					1	
6										
7										
<i>Garnet</i>										
1										
2	30	6					40	18	28	13
3	1	1						1	9	3
4									2	
5										
6										
7										

^a P = Bunted culms partially infected plants.^b C = Bunted culms completely infected plants.^c Numbers refer to enumerated designations in column 1, table 2.

tion occurred in the spikes of Reward infected with collections 1 and 5, but when infected with collections 2 and 3 considerable elongation resulted. Kota infected with collection 1 showed little or no spike elongation, but when infected with either collections 2 or 5, considerable spike elongation occurred. Infected spikes of partially infected plants showed as much spike elongation as the spikes of completely infected plants.

The marked elongation of the spike of Little Club, when infected with bunt, is in agreement with other results reported on club wheats for this character (2, 6, 14, 15). No indication of a consistent shortening of infected spikes, as reported by Barrus (1) and Vilkaitis (13) was obtained. Potter and Coons (8) report differences between the two species of *Tilletia* in regard to general shape of infected heads. In the present investigation differences were noted but were not found to be consistent for a single species, which agrees with the results of Rodenhiser (9). Smith (11) reports that heads of Martin wheat, infected with physiologic form T_2 , were of a different shape than those infected with form T_3 . In the present study indications were also obtained that different collections of bunt react differently on the same variety. In the case of Reward, infection with collections 2 and 3 resulted in a much greater degree of spike elongation than when infected with collections 1 and 5.

The results of this investigation indicate that the degree of spike elongation of infected heads is influenced by both the collection or physiologic form of bunt used, and the host.

SHAPE OF BUNT BALLS

Barrus (1) observed that the shape of bunt balls of infected heads were smaller in all dimensions than the kernels from normal heads. Potter and Coons (8) state that the sori of *Tilletia levis* are longer and narrower than those of *T. tritici*. Rodenhiser (9) reports differences in the general shape of bunt balls of the two species of *Tilletia* on different hosts, but these differences were not constant for a single species. Feucht (3) states that with both species of *Tilletia* he obtained swollen as well as slender, almost elongated, oval balls; and that their general shape seemed to be more determined by variety than by any inherent tendency of the fungus. Smith (11) found differences in the bunt ball shape of two physiologic forms of *T. tritici* on Martin wheat, one of which produced a shrivelled angular ball, while the ball of the other form was rounder in shape.

EXPERIMENTAL RESULTS

The bunt balls were classified into 5 classes for shape, 1 being round and 5 long and narrow (Fig. 2). In table 4 the data give the total length in centimeters of 10 kernels of the 6 varieties and the mean class for bunt-ball shape for each variety of wheat infected with each collection of bunt.

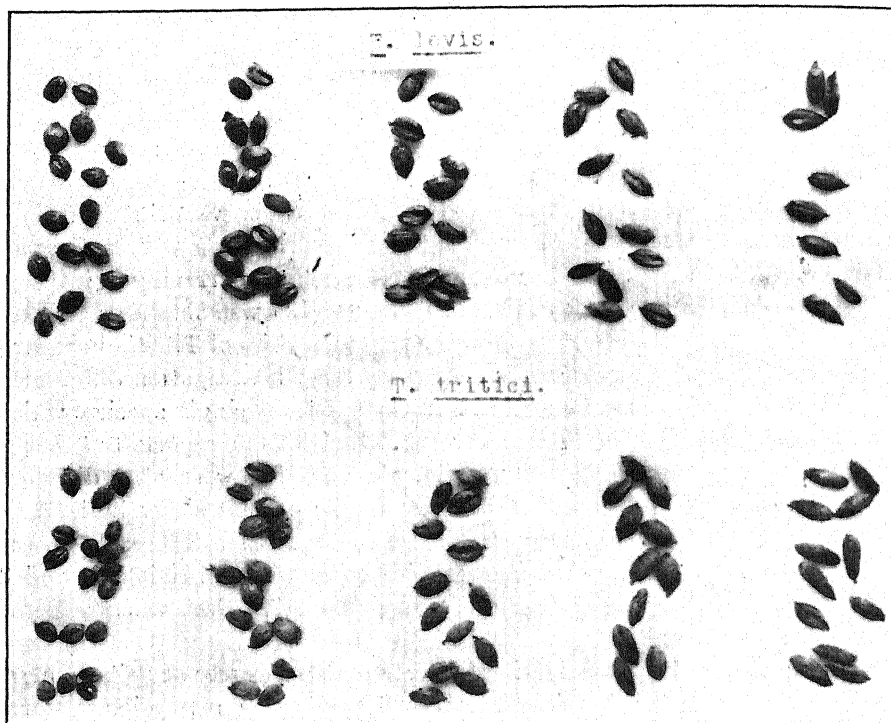


FIG. 2. Great variability found in the size and shape of the bunt balls of both species of *Tilletia*.

No difference was noticed in the bunt-ball shape of partially infected plants and those of completely infected plants for any variety infected with any collection. The bunt balls within a single spike showed considerable variation in shape and size. Little difference existed in the average bunt ball-shape for the different collections on any single variety.

The data in table 4 show that the average bunt-ball shape of collection 1, *Tilletia tritici*, tended to be rounder than those from the 4 collections of *T. levis*. This cannot be taken as conclusive evidence that the bunt balls of *T. tritici* tend to be smaller than those of *T. levis*, for only one collection of *T. tritici* was used. Since the range in shape of the bunt balls of *T. tritici* was similar to that for *T. levis* (Fig. 2), it is impossible to tell by the shape of a bunt ball to which of the two species it belongs. In figure 2 is shown the great variability found in the size and shape of the bunt balls of both species of *Tilletia*. The data show that the bunt balls on the varieties of Pentad, Hope and Kota having the long normal kernel, tended to be longer than those produced on the varieties of Reward, Little Club and Garnet, which have shorter normal kernels.

TABLE 4.—*Total length in centimeters of ten kernels from, and average bunt-ball shape for each bunt collection on Reward, Little Club, Pentad, Hope, Kota, and Garnet spring wheats*

Variety	Total length of 10 kernels in centimeters	Classes for average bunt ball shape ^a for the different collections of bunt				
		1	2	3	4	5
Reward	6.3	2	3	3	...	3
Little Club	6.1	2	2.5	2.5	2.5	...
Pentad	7.1	4	4	4.5	4	...
Hope	7.3	2.5	3.5
Kota	7.0	3	4	...	4	...
Garnet	6.1	1.5	2	2.5

^a 1=round to 5=long and narrow.

That certain physiologic forms produce bunt balls of different shape is clearly indicated by the work of Smith (11), who obtained differences in bunt-ball shape between 2 physiologic forms of *Tilletia tritici*, on Martin wheat. There appears to be a general tendency for the shape of the bunt balls produced by *T. tritici* to be slightly rounder than those produced by *T. levis*, which was previously reported by Potter and Coons (8). However, this general tendency found in the present study cannot be taken as being conclusive, for only one collection of *T. tritici* was tested. As previously pointed out, it is impossible to distinguish between the bunt balls of the two species, for both have a similar range for size and shape. The results of this study strongly support the statement of Feucht (3) to the effect that the general shape of bunt balls is determined more by variety than by any inherent tendency of the fungus.

ASSOCIATION BETWEEN CHARACTERS

Tests for independence were calculated in order to determine any possible relationships that may exist between length of culm, spike elongation, and bunt-ball shape. The method of analysis used was that described by Fisher (4). Tests were calculated for both the partially and completely infected plants with each variety for all collections of bunt with which they were inoculated. Significant associations were obtained only in 3 instances: Reward, infected with collection 2, showed a positive association between spike elongation and bunt-ball shape, for the completely infected plants; Little Club, infected with collection 1, showed a positive association between length of culm and spike elongation, for completely infected culms; Little Club, infected with collection 1, also showed a positive association between

length of culm and bunt-ball shape. In these 3 instances no association was found in the case of the bunted culms of the partially infected plants. The data for the other varieties indicate that, in general, there are no associations between bunt-ball shape, spike elongation, or length of culm in the varieties tested, and for the collections of bunt used.

SUMMARY

The effect of bunt on the morphology of the wheat plant was studied with the 6 spring wheat varieties: Reward, Little Club, Pentad, Hope, Kota, and Garnet, inoculated separately with one collection of *Tilletia tritici* and four of *T. levis*.

Infection with either species of *Tilletia* resulted in a reduction of the culm length of the wheat plant. However, no significant difference was obtained in the degree of reduction produced by the two species.

The different collections reacted somewhat differently on the different wheat varieties.

The stunting of inoculated nonbunted plants indicates that nearly all plants became infected when inoculated and kept under favorable conditions for the fungus to develop.

In general, no difference was detected in the culm length of the bunted culms of partially bunted plants and those of the completely bunted plants.

Spike elongation of bunt-infected heads occurred only in the more susceptible varieties: Reward, Little Club, and Kota. The susceptible varieties tended to react somewhat differently to the different collections used.

The general shape of the bunt balls was determined more by the variety than by any inherent tendency of the fungus.

The range in size of the bunt balls for all collections was the same. However, those of a single collection of *T. tritici* tended on the average to be smaller and rounder than those of *T. levis*.

No general association was found to exist between culm length, spike elongation and bunt-ball shape.

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FACTOR RELATIONS IN WHEAT FOR RESISTANCE TO PUCCINIA GRAMINIS TRITICI, PUCCINIA GLU- MARUM AND ERYSIYPHE GRAMINIS¹

K. W. NEATBY²

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INTRODUCTION

Three decades have elapsed since Biffen (2, 3) first demonstrated that resistance to stripe rust of wheat was inherited according to Mendelian laws. During this time an enormous amount of information concerning the genetics of disease resistance in plants has been accumulated. Surprisingly little attention, however, has been devoted to the question of the genetic relationship between resistance to different diseases. Two causes, at least, underlie this neglect. In the first place, most of the work has been prompted by local needs. For a particular crop in any one locality there are commonly one or, perhaps, two diseases of outstanding importance. The most urgent problems have, not unnaturally, received the first attention. In the second place, most investigators in this field have sought to determine the actual number of genes concerned in the inheritance of the reaction to the disease in which they were interested. This necessitates the use of F_3 material, and the small amount of available seed usually limits the study to one, or at most two, diseases. A few cases of genetic linkage have been reported, and these will be briefly reviewed.

Hayes, Aamodt, and Stevenson (7) and Goulden and Neatby (4) have made certain observations that suggest a linkage between susceptibility to black chaff and resistance to *Puccinia graminis tritici* in wheat crosses. This relationship has been studied further by Hayes *et al.* (8) and Ausemus (1). It is not certain, in the opinion of the present writer, that the relationship is due to genetic linkage. It may be that rust-infected plants do not provide a suitable medium for the development of black chaff, and that in the absence of rust infection they might be susceptible to black chaff. This possibility has not been adequately considered.

Hayes *et al.* (8) found some indication of linkage between resistance to *Puccinia triticina* and resistance to *P. graminis tritici*.

In a cross between the wheat varieties Federation and Thew, Waterhouse (13) found resistance to *Puccinia triticina* and resistance to *Erysiphe graminis* to be completely linked.

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² Fellow of the National Research Council.

In a study of the inheritance of resistance to bunt of wheat in the cross Kota \times Garnet, Kilduff (9) found resistance to *Tilletia laevis* (*T. foetens*) to be correlated with resistance to *T. tritici*.

Reed (12) made a study of the inheritance of resistance to *Ustilago avenae* and *U. levis* in crosses between the oat varieties Black Mesdag and Hulless. The results indicated that the resistance of Black Mesdag to the two smuts was inherited as a simple Mendelian dominant. Welsh (14) also found resistance to *U. avenae* and to *U. levis* to be closely correlated in the cross Victory \times (Minota-White Russian \times Black Mesdag).

Several cases of independent inheritance of the reaction to different plant diseases have been reported. These will not be reviewed here. A list of references is given by Hansen (6).

The following pages contain a report of the preliminary results of a study of the genetic relationship between the reactions to various fungous diseases of wheat.

MATERIALS

The present study is concerned with the genetic relationship between the reactions of wheat to *Puccinia graminis tritici* Eriks. and Henn., *P. glumarum tritici* Eriks., *Erysiphe graminis* DC., and pigmentation of the straw. The various relationships have been studied in 3 different wheat crosses, namely: H-44-24 \times Marquis, Marquillo \times H-44-24 and Garnet \times Double Cross. The wheat material was studied in the sixth generation, and was unselected except that lines segregating for seedling reaction to *P. graminis* forms 21 and 36 were eliminated in F_4 . The origin of the parental varieties, and the results of studies with *P. graminis* alone, have been reported in an earlier publication (10).

All the data on *Puccinia graminis* were obtained at Winnipeg, Canada, in the years 1929 and 1930. The rest of the work was done at Cambridge, England, in 1933-34.

The reactions to *Puccinia glumarum*³ were determined by means of seedling tests made in the greenhouse. The tests with *P. graminis* were made in the field (mature-plant reactions) and in the greenhouse (seedling reactions). The mature-plant resistance of the variety H-44-24 to *P. graminis* is inherited independently of the seedling reaction. The two reactions are, therefore, considered separately in relation to the other characters.

The reactions to *Erysiphe graminis* were studied both in the greenhouse and in the field. The greenhouse reactions were determined by means of seedling tests. A heavy natural epidemic made an accurate field classification possible. The field notes were taken shortly after the wheat had come into head. There was no marked resistance to *E. graminis* in the cross Gar-

³ The writer is indebted to Mrs. F. C. Bawden, School of Agriculture, Cambridge, for the cultures of *P. glumarum* used.

net \times Double Cross so, naturally, no relationships could be established. The variety H-44-24 proved highly resistant both in the field and in the greenhouse. The lines of the crosses H-44-24 \times Marquis and Marquillo \times H-44-24 were, with few exceptions, definitely resistant, susceptible, or segregating. The seedling reactions determined in the greenhouse agreed very closely with the field reactions, so that the two will not be considered separately.

Under the conditions prevailing in the field at Cambridge in 1934, H-44-24 was characterized by a deep red coloration of the straw. In the H-44-24 \times Marquis lines a classification according to the presence or absence of red pigment was very simple. The lines were definitely pigmented, nonpigmented, or segregating. In Marquillo \times H-44-24 all the lines were more or less pigmented. In Garnet \times Double Cross the distinction between pigmented and nonpigmented lines was fairly clear, though a few doubtful cases were encountered.

Seedling reactions to each of the two rusts were classified as resistant (R), moderately resistant (MR), semiresistant (SR), moderately susceptible (MS) and susceptible (S). In the case of the trials with *Puccinia graminis*, individual lines were marked R, MR, etc., according to the particular reaction. It was not considered necessary to record the reaction of individual seedlings, as most segregating lines had been previously discarded. The seedlings of each line were classified individually for their reactions to *P. glumarum*, and a mean value calculated for each line. For the purpose of calculating the means, the numerals 1 to 5 were substituted for the symbols R, MR, etc. The tests with *P. graminis* were not duplicated except in certain doubtful cases. The tests with *P. glumarum* were duplicated in the cross H-44-24 \times Marquis, but not in the other two crosses.

The chief characteristics of the parental varieties are recorded in table 1. It is necessary to remember, in connection with the reactions to *P. graminis*, that "field reaction" refers to the reaction under field conditions of plants

TABLE 1.—Description of the parental varieties in regard to the characters concerned

Variety name	<i>Puccinia graminis</i> (field)	<i>Puccinia graminis</i> (greenhouse)	<i>Puccinia glumarum</i> (greenhouse)	<i>Erysiphe graminis</i>	Straw color
Marquis	S	S(forms 36 & 52)	MS	S	White
H-44-24	R	R(forms 36 & 52)	SR	R	Red
Marquillo	MR	MS(forms 36 & 52)	R	S	Red
Double Cross ..	MR	R(forms 21 & 35)	R	S	Pale red
Garnet	S	S(forms 21 & 35)	SR	S	Red

approaching maturity. Thirty or more physiologic forms were involved in the field epidemics. "Greenhouse reaction" refers to the reaction of seedlings under greenhouse conditions.

RESULTS WITH H-44-24 × MARQUIS

Puccinia glumarum and Field Reaction to *P. graminis*

The variety H-44-24 is extremely resistant to *Puccinia graminis* under field conditions at Winnipeg. Judged by the seedling reaction in the greenhouse, it varies from high resistance to moderate susceptibility, depending on the particular physiologic form used. The field, or mature-plant, resistance is inherited as a simple Mendelian dominant in the cross H-44-24 × Marquis and is independent of the seedling reaction (5, 10).

In this cross 114 lines were tested in duplicate with culture 41 (Botany School numbering) of *Puccinia glumarum*. Since it was not possible to maintain a constant temperature in the greenhouse, the two trials were conducted at different times. The first series was inoculated on the 5th of March, and the second on the 19th of April. Mean values of each line in each test were calculated. The mean values obtained in the duplicate trials were closely correlated, r being equal to .87. This indicates that the classification was fairly accurate. The distinction was, for the most part, between semi-resistance and moderate susceptibility; consequently, a certain amount of error in classification was inevitable.

In table 2 the wheat strains are arranged according to their field reaction to *Puccinia graminis* and seedling reaction to *P. glumarum*. The means of the *P. glumarum* reactions are based on the average value of the two trials. A close relationship between resistance to the two organisms is evident. It appears that the gene concerned in the difference between H-44-24 and Marquis in regard to their field reactions to *P. graminis* also plays the chief rôle in the inheritance of resistance to *P. glumarum*.

TABLE 2.—Lines of the cross H-44-24 × Marquis arranged according to seedling reaction to *Puccinia glumarum* and field reaction to *P. graminis*. (*P. glumarum* means based on the average of duplicate trials)

<i>Puccinia graminis</i>	<i>Puccinia glumarum</i>						
	a2.00	2.01-2.50	2.51-3.00	3.01-3.50	3.51-4.00	4.01-4.50	4.51-5.00
Resistant	1	6	14	5	5		
Susceptible				2	18	11	17
Segregating		2	3	9	12	6	2

^a 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

TABLE 3.—*Lines of the cross H-44-24 × Marquis arranged according to seedling reaction to Puccinia glumarum and field reaction to P. graminis. (P. glumarum means based on the second trial)*

<i>Puccinia graminis</i>	<i>Puccinia glumarum</i>						
	2.00	2.01–2.50	2.51–3.00	3.01–3.50	3.51–4.00	4.01–4.50	4.51–5.00
Resistant	4	8	10	6	3		
Susceptible					20	5	23
Segregating		1	4	5	17	3	5

^a 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

An even closer relationship is evident if the mean values of the second trial to *Puccinia glumarum* are used. The explanation of this is probably to be found in the fact that the conditions in the greenhouse for the development of *P. glumarum* were better in April, when the second trial was made, than in March. The results are given in table 3.

It is clear from the data presented that field resistance to *Puccinia graminis* is very closely linked with the seedling reaction to *P. glumarum* in the cross H-44-24 × Marquis. Probably the same gene is responsible for the inheritance of resistance to the two organisms. It is, of course, possible that the relationship is due to the close linkage of two different pairs of genes. This possibility cannot be dismissed or confirmed without more extensive data.

The variability of the reactions to *Puccinia glumarum* within the resistant and susceptible classes of *P. graminis* is probably due, in part, to genetic causes. The evidence for this conclusion is derived from a study of the relationship between the duplicate trials with *P. glumarum* within the resistant and susceptible classes of *P. graminis*. If the variation in these two classes were random, there should be no correlation between duplicate trials. In the group resistant to *P. graminis* the correlation between duplicate trials with *P. glumarum* was $r = .46$ ($t = 2.64$). The three lines that gave mean reactions to *P. glumarum* of over 3.50 (see table 3) in the second trial were not included in the calculation of the correlation coefficient. They probably were wrongly classified as R to *P. graminis*. In the group susceptible to *P. graminis* the correlation between duplicate trials with *P. glumarum* was $r = .56$ ($t = 4.58$). It is evident, therefore, that there are one or more genes modifying the reaction to *P. glumarum* in the groups resistant and susceptible to *P. graminis*.

It appears from the above data that the inheritance of the seedling reaction to *Puccinia glumarum* form 41 and the field reaction to *P. graminis* is

mainly controlled either by one gene or by two closely linked genes. This result is significant in relation to certain wheat-breeding problems. It is remarkable that, although the field reaction to *Puccinia graminis* is not genetically associated with the seedling reaction to this organism (except in a very limited way, to be noted later), yet it is associated with the seedling reaction to *P. glumarum*. The most significant feature of this result is the provision of a method whereby selection for field resistance to *P. graminis*, in crosses involving H-44-24, can be made by a simple test in the greenhouse. The test is of particular value to breeders who cannot rely on an annual outbreak of stem rust in their experimental plots.

Puccinia graminis Form 36 and *Erysiphe graminis*

A somewhat intricate relationship between the reactions to *Erysiphe graminis* and form 36 of *Puccinia graminis* is evident in tables 4 to 7. In table 4 there is a deficiency of lines susceptible to both organisms; the deficiency, however, is not statistically significant. Consideration of only those lines characterized by mature-plant resistance to *P. graminis* (table 5) is very suggestive. The 5 lines susceptible to *E. graminis* are all resistant or moderately resistant to *P. graminis* form 36. So few lines are available for this comparison that the result cannot be considered conclusive. An interesting check on this result is afforded by a group of lines of the same cross selected for field resistance to *P. graminis*. Reactions to form 36 and *E. graminis* also were determined for these lines. The result is set forth in table 6. The agreement between the two independent results is very close,

TABLE 4.—Distribution of H-44-24 × Marquis lines according to the reactions to *Erysiphe graminis* and form 36 of *Puccinia graminis*

<i>Erysiphe graminis</i>	<i>P. graminis</i> form 36			
	R	MR	SR	S
Resistant	8	12	17	9
Susceptible	9	3	7	2

TABLE 5.—Lines arranged according to the same characters as in table 4 but including only those lines possessing the field resistance of H-44-24 to *Puccinia graminis*

<i>Erysiphe graminis</i>	<i>P. graminis</i> form 36			
	R	MR	SR	S
Resistant	4	2	3	2
Susceptible	2	3

and leaves little doubt regarding the reality of the relationship. The two sets of data are combined in table 7.

TABLE 6.—Lines arranged according to the same characters as in table 5, but derived from a different set of H-44-24 × Marquis material, and all possessing the field resistance of H-44-24 to *Puccinia graminis*

<i>Erysiphe graminis</i>	<i>P. graminis</i> form 36			
	R	MR	SR	S
Resistant	8	7	2	4
Susceptible	6	4

TABLE 7.—Tables 5 and 6 combined

<i>Erysiphe graminis</i>	<i>P. graminis</i> form 36			
	R	MR	SR	S
Resistant	12	9	5	6
Susceptible	8	7

The situation with regard to the three characters concerned is somewhat complex. We know from previous work (5) that there are two genes concerned in the inheritance of the seedling reaction to form 36. Since there are more than twice as many lines resistant to *Erysiphe graminis* as there are susceptible, in all probability more than one gene is involved in the inheritance of the reaction to this organism. Field resistance to *Puccinia graminis* in this cross is inherited as a simple Mendelian dominant. The relationship observed could be conditioned by the operation of four genes, as follows: R, a gene for field resistance to *P. graminis*; X, a gene for resistance to form 36; Y, a gene for resistance to form 36 and susceptibility to *E. graminis* which is expressed only in the presence of R; and Z, a gene for resistance to *E. graminis* which is epistatic to Y. The expected phenotypes on the basis of this theory are given in table 8.

The theory proposed is in accordance with the facts as far as the various combinations of characters are concerned. It explains why all lines resistant in the field to *Puccinia graminis* and susceptible to *Erysiphe graminis* are resistant to form 36. If this explanation be correct, the segregation for reaction to form 36 should be bifactorial in the field rust-resistant class, and monofactorial in the field rust-susceptible class (Y being nonfunctional in the latter). That the segregation is quite different in the two classes is indicated by the data in table 9.

TABLE 8.—*Expected phenotypes in the cross H-44-24 × Marquis in regard to the field reaction to Puccinia graminis, the seedling reaction to P. graminis form 36, and the reaction to Erysiphe graminis according to the theory proposed^a*

Phentotype	Field reaction to <i>P. graminis</i>	Seedling reaction to form 36	Reaction to <i>E. graminis</i>
RXYZ	resistant	resistant	resistant
RXYz	"	"	susceptible
RXyZ	"	"	resistant
RxYZ	"	"	"
RXyz	"	"	"
RxYz	"	"	susceptible
RxyZ	"	susceptible	resistant
Rxyz	"	"	"
rXYZ	susceptible	resistant	"
rXYz	"	"	susceptible
rXyZ	"	"	resistant
rxYZ	"	susceptible	"
rXyz	"	resistant	susceptible
rxYz	"	susceptible	"
rxYz	"	"	resistant
rxyz	"	"	susceptible

^a Heterozygous lines are not considered. See also the text and tables 4 to 7.

TABLE 9.—*Lines of the cross H-44-24 × Marquis arranged according to the seedling reaction to Puccinia graminis form 36 and the field reaction to P. graminis*

Field reaction	<i>P. graminis</i> form 36			
	R	MR	SR	S
Resistant	17	3	8	4
Susceptible	9	13	14	10

Apart from the two relationships described, no linkages were evident between any two of the four characters concerned. The presence or absence of red pigment in the straw appeared to be independent of the reactions to the three diseases.

MARQUILLO × H-44-24

Parental Varieties

The variety Marquillo is moderately resistant to *Puccinia graminis* under field conditions. It is also resistant in the seedling stage, under greenhouse conditions, to certain physiologic forms of *P. graminis*. The inheritance of seedling and of mature-plant reactions is governed by the same genes in

crosses between Marquillo and susceptible varieties. In other words, Marquillo has no 'mature-plant' resistance in the sense that H-44-24 has (11). In the seedling stage, and probably under field conditions, Marquillo is highly resistant to *P. glumarum*. In view of these facts it is natural to expect the genetic behavior of resistance to the two rusts to be very different in this cross from that found in H-44-24 × Marquis.

Puccinia graminis (Field Reaction) and *P. glumarum*

In regard to the genetic relationship between resistance to *P. graminis* and *P. glumarum* some interesting facts have come to light. A total of 224 lines of this cross were used. One lot of 113 lines was inoculated with form 22 of *P. glumarum*, and the remainder with form 41. The two trials gave essentially identical results when considered in relation to the *P. graminis* reactions; consequently, only the data obtained with form 22 are given. Lines that segregated for reaction to *P. graminis* are not included in the tables. The *P. glumarum* tests were not duplicated.

TABLE 10.—The lines of the cross Marquillo × H-44-24 arranged according to the seedling reaction to *Puccinia glumarum* and the field reaction to *P. graminis*

<i>P. graminis</i> (field)	<i>P. glumarum</i> form 22				
	1.00 ^a	1.01– 2.00	2.01– 3.00	3.01– 4.00	4.01– 5.00
Resistant	13	9	13	8	2
Semiresistant	3	2	4
Susceptible	9	4	4	10

^a 1.00 = R, 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

In table 10 the lines are distributed according to the field reaction to *Puccinia graminis* and greenhouse reaction to *P. glumarum*. As in the case of H-44-24 × Marquis, each seedling was classified for its reaction to *P. glumarum* and the mean calculated for each line. It is evident from the data in table 10 that the high resistance (1.00) to *P. glumarum*, characteristic of Marquillo, is not correlated with the field reaction to *P. graminis*. However, the semiresistance (3.00) of H-44-24 is definitely associated with field resistance to *P. graminis*. This is to be expected in view of the result obtained in H-44-24 × Marquis (table 2). It follows from this result that in Marquillo × H-44-24, as in H-44-24 × Marquis, selection for field resistance to *P. graminis* can be based on seedling tests in the greenhouse. Lines semiresistant to *P. glumarum* in the greenhouse will possess the high field resistance of H-44-24 to *P. graminis*. The results of previous studies (11) have

shown that the seedling reaction to form 21 of *P. graminis* may be used as a basis for selection for the field resistance of Marquillo.

Puccinia graminis Form 52 and *P. glumarum* Form 22

In the classification according to the seedling reaction to *P. graminis*, the reaction of individual seedlings was not recorded. The lines were classified as resistant, moderately resistant, etc., or segregating. Of the 113 lines classified for their reaction to *P. glumarum*, 11 lines were heterozygous for the *P. graminis* reaction, and the reactions of 6 were not determined. This leaves a total of 96 lines available for comparison. The relationship between the two reactions is illustrated in table 11. The lines fall into two well-

TABLE 11.—The lines of the cross Marquillo \times H-44-24 arranged according to the seedling reactions to *Puccinia glumarum* and *P. graminis* form 52

<i>P. graminis</i> form 52	<i>P. glumarum</i> form 22				
	1.00 ^a	1.01– 2.00	2.01– 3.00	3.01– 4.00	4.01– 5.00
Resistant	9	8	9	7
Moderately resistant	2	...	5	4	15
Semiresistant	17	3
Susceptible	12	5

^a 1.00 = R, 2.00 = MR, 3.00 = SR, 4.00 = MS, 5.00 = S.

defined groups; one more or less resistant to *P. graminis* and tending towards susceptibility to *P. glumarum*, and the other more or less susceptible to *P. graminis* and resistant or moderately resistant to *P. glumarum*. It is also possible that the difference between resistance and moderate resistance to *P. graminis* may be associated with the difference between moderate susceptibility and susceptibility to *P. glumarum*.

The actual number of genes concerned in the inheritance of reaction to these two rusts in this cross is not known. Since Marquillo is highly resistant, and H-44-24 semiresistant to *Puccinia glumarum*, and 22 of the 96 lines were definitely susceptible, it is certain that at least two genes are in operation. Judging from the variety of the types of infection by *P. graminis*, also, it is almost certain that two genes, at least, are involved. In view of these facts it is extremely improbable that the relationship observed can be explained on the basis of genetic linkage. It is probable that the genetic constitution that renders a plant resistant to *P. graminis* predisposes it to attack by *P. glumarum*. Likewise, plants genetically susceptible (more or less) to *P. graminis* are resistant or moderately resistant to *P. glumarum*.

In this cross there was no evidence of linkage of the other characters concerned, with the possible exception of *Erysiphe graminis* and the two rusts. There was a tendency for the lines susceptible to *E. graminis* to be concentrated in the MR and SR groups of *Puccinia graminis* form 52. However, the association was only just on the borderline of statistical significance.

GARNET × DOUBLE CROSS

Puccinia graminis Form 35 and *P. glumarum* Form 41

In this cross, resistance to *P. glumarum* is inherited independently of the field reaction to *P. graminis*. The reaction to *P. glumarum* is, however, closely associated with the greenhouse reaction to *P. graminis* form 35. The data illustrating this relationship are given in table 12. The susceptibility of Garnet to form 35 was not recovered in this cross, so that the *P. graminis* classification rested on the distinction between R and SR. The *P. glumarum* tests were made as in the other two crosses; but, as the majority of the lines were definitely resistant or susceptible, the remainder are grouped together in the table as SR and segregating. In all, 115 lines were tested; 24 were heterozygous for reaction to form 35, and these are excluded from the table. Neither of the tests was duplicated.

TABLE 12.—*The lines of the cross Garnet × Double Cross arranged according to their reactions to Puccinia graminis form 35 and P. glumarum form 41*

<i>P. graminis</i> form 35	<i>P. glumarum</i> form 41		
	R	SR & Seg.	S
Resistant	2	13	24
Semi-resistant	33	14	5

The relationship between the reactions to the two rusts is similar to that found in the cross Marquillo × H-44-24 (table 11); that is to say, resistance to one rust is closely associated with susceptibility to the other. The variety Double Cross (Marquillo × Marquis-Kanred) inherits its high resistance to *P. glumarum* from Marquillo. It seems, therefore, that the genes concerned in the resistance of Marquillo to *P. glumarum* are responsible for its susceptibility to certain physiologic forms of *P. graminis*. In table 12 there are two lines resistant to both rusts and five susceptible to both. This suggests that the relationship might be due to genetic linkage, and the lines giving similar reactions to both rusts would then represent the cross-over classes. However, as the susceptibility of Garnet to form 35 was not recovered (except in segregating lines) in spite of the fact that over 900 F₄ lines were tested, at least three genes, and probably more, must be in operation. The

reactions to *P. glumarum* likewise involve more than one gene, since Garnet is semiresistant and Double Cross highly resistant and 29 lines were definitely susceptible. In view of these facts it is unlikely that the relationship is due to genetic linkage. More probably it is due to the genes governing resistance to one rust being responsible for susceptibility to the other. If this be the case, the lines classified as resistant to both and those susceptible to both must be wrongly classified. It is unlikely that the errors were made in the *P. glumarum* classification, as the distinction between the R and S classes was quite clear. The distinction between the R and SR classes of *P. graminis*, however, was not always clear and errors were probably made. Unfortunately, form 35 of *P. graminis* has been lost, so that the result cannot be checked.

Puccinia graminis Form 21 and Straw Pigmentation

The variety Double Cross inherits immunity from *P. graminis* form 21 from Kanred. Crosses with other varieties show that it also possesses the Marquillo genes concerned in high resistance to form 21. The Kanred immunity is inherited independently of the Marquillo resistance (10).

TABLE 13.—The lines of the cross Garnet × Double Cross arranged according to the pigmentation of the straw and reaction to *Puccinia graminis* form 21

Straw color	<i>P. graminis</i> form 21			
	O ^a	R	SR	S
Red	25	4	6	12
White	25	4	14	1

^a O = immune.

In table 13 the Garnet × Double Cross lines are arranged according to their reactions to form 21 and straw pigmentation. Of the 13 lines susceptible to form 21, only one has white straw. The reaction of this line to form 21 has been checked and its susceptibility confirmed. It is possible that an error in the classification of the straw color may have been made, and it also is possible that the line may be a result of natural crossing in F_2 with a white-straw variety. In any case there can be little doubt that the characters are genetically associated. The χ^2 test of independence gives a P value of less than .01. In the calculation of χ^2 the O and R classes were combined.

If the one white susceptible line is disregarded, the results can be explained by assuming that two genes are involved in the inheritance of straw color, and that the presence of one of them is essential to susceptibility to

form 21. According to this theory, purple lines may be either resistant or susceptible, while white lines are all more or less resistant.

Apart from the two cases of association described, the characters concerned appear to be inherited independently in the cross Garnet \times Double Cross.

GENERAL DISCUSSION

The results of the investigation described in this paper are interesting in two respects, firstly; from the point of view of the production of disease-resistant varieties, and secondly; in regard to the nature of disease resistance in plants.

If reactions to all the important diseases of wheat were inherited independently, the development of varieties resistant to all would be merely a question of large-scale operation (that is, of course, assuming that no one organism is capable of attacking all varieties of the mutually interfertile species). By making appropriate crosses and studying a sufficiently large number of lines, it would be possible to combine in one variety resistance to all the fungous diseases concerned. It is evident from the results described in this paper that the reactions to different diseases may be correlated in inheritance. When resistance to two or more diseases is correlated, the breeding problem is simplified. An example of this is provided by the relationship between resistance to *Puccinia glumarum* and field resistance to *P. graminis* in the cross H-44-24 \times Marquis. The direct association between resistance to one disease and susceptibility to another may render the combination of resistance to both in one variety impossible, at least for that particular cross. An example of such a relationship is provided by the results obtained with *P. graminis* form 52 and *P. glumarum* form 22 in the cross Marquillo \times H-44-24. It is not necessary, however, to suppose that a relationship established in one cross will hold in another; in fact, it frequently does not. This is not surprising, since, in the case of *P. graminis*, for example, several sources of resistance, due to different genes, are available.

In regard to the fundamental nature of disease resistance, some of the results presented are of interest. If, as the data indicate, genes responsible for the inheritance of resistance to one disease may be concerned in susceptibility to another, any theory offered to explain the nature or cause of resistance to one must, at the same time, explain the nature or cause of susceptibility to the other. The cases of the relationship between resistance to *Puccinia graminis* and susceptibility to *P. glumarum* in H-44-24 \times Marquillo and in Garnet \times Double Cross are, in the writer's opinion, most easily explained by supposing that the internal condition of a plant that predisposes it to attack by *P. graminis* renders it resistant to *P. glumarum*. The possibility of the relationships described being due to genetic linkage cannot be entirely dismissed; but it is extremely improbable that genetic linkage will

explain the results, particularly when more than one pair of genes is concerned in the inheritance of the individual characters.

SUMMARY

In the cross H-44-24 \times Marquis the seedling reaction to *Puccinia glumarum* was found to be closely associated with the mature-plant reaction to *P. graminis*. In the group of lines with mature-plant resistance to *P. graminis* all lines susceptible to *Erysiphe graminis* were resistant or moderately resistant to *P. graminis* form 36, and all lines susceptible or semiresistant to form 36 were resistant to *E. graminis*.

In Marquillo \times H-44-24 semiresistance to *P. glumarum* was found to be associated with the mature-plant resistance of H-44-24 to *P. graminis*. Resistance in the seedling stage to form 52 of *P. graminis* was associated with susceptibility to *P. glumarum*, and, likewise, susceptibility to form 52 was associated with resistance to *P. glumarum*.

In Garnet \times Double Cross the relationship between the seedling reactions to *P. graminis* form 35 and *P. glumarum* was similar to that found in Marquillo \times H-44-24 in regard to *P. graminis* form 52 and *P. glumarum*. Susceptibility to *P. graminis* form 21 was associated with red pigmentation of the straw.

It is concluded that the relationships described are more probably due to a pleiotropic effect of the genes concerned than to genetic linkage.

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UNIVERSITY OF ALBERTA,

EDMONTON, ALBERTA, CANADA.

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PERSISTENCE OF ERWINIA AMYLOVORA IN CERTAIN INSECTS

P. A. ARK AND H. EARL THOMAS

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Soon after the discovery of the cause of transmissible diseases of animals the medical profession was able to place a certain amount of responsibility in dissemination on insects, such as flies, fleas, cockroaches, bed bugs, etc. The feeding habits of many species of insects resulted in the spread of such diseases as typhoid fever and cholera (12). What was true for pathogens of animals became applicable to microorganisms causing diseases in plants. As early as 1891, Waite (14) directed the attention of plant pathologists to the fact that the fire-blight organism was spread by various insects: bees, flies, etc. Since that time a large number of diseases has been directly or indirectly connected with insects. However, experiments and observations were for the most part concerned with the external or mechanical transmission of diseases until quite recently, when some consideration has been given to the fate of microorganisms in the internal organs of insects.

Rand and Pierce (11), considering the internal transmission of pathogenic microorganisms, distinguish mechanical internal transmission in which the pathogens stay unchanged after passage through the alimentary tract without undergoing any appreciable multiplication or development within the insect body (*Bacillus pestis*), and biological internal transmission in which case the pathogens are able to multiply in insects and thus persist for a considerable time (*B. typhosus*, *B. tracheiphilus*). Both of these types of transmission have been shown for bacterial pathogens of plants and animals (1, 2, 8, 9, 10).

In order to obtain information as to the possible rôle played by some insects in biologic transmission of the fire-blight organism, the writers undertook an investigation under laboratory conditions employing *Drosophila melanogaster* (fruit fly), *Musca domestica* (house fly), *Lucilia sericata* (green bottle fly) and *Apis mellifica* (honey bee). Flies of these and other kinds are commonly found on the holdover cankers and in the blossoms of pear trees. Fruit-fly larvae and adults were found in decaying pear and apple fruits in Sonoma County, California, in January, 1935.

Drosophila flies were reared in sterile, cooked apple mash. The parent culture was procured from the Department of Genetics of this University. As soon as the larvae of *D. melanogaster* were plentiful they were transferred aseptically into another container, with apple medium contaminated with a 48-hour-old culture of *Erwinia amylovora* (Burrill) Winslow *et al.* Larvae were allowed to feed on the contaminated medium for 24 hours,

following which interval one lot of larvae was cultured in broth, while another lot was transferred into a fresh sterile apple medium for pupation. To culture the first lot, larvae were removed from the contaminated medium and sterilized with a 1 to 500 solution of HgCl_2 or 3 per cent Lysol solution for 3 minutes. After this the larvae were dropped into sterile nutrient beef broth of pH 6.9, crushed by means of a flamed flat needle and incubated at 28° C. Two kinds of control were included in this test. One consisted of inoculum from the flask of apple medium plus *E. amylovora*, in order to be certain of the viability of *E. amylovora*, and the other was surface-sterilized and washed, uncrushed larvae that were allowed to remain in broth at 28° C. for 24 hours only. Any growth that appeared was plated out and diagnosed for *E. amylovora* with the pathogenicity test on fire-blight susceptible plants. The results of this first experiment were as follows: Out of 100 larvae 44 were positive for *E. amylovora*. The first control was positive for *E. amylovora*, while the second remained clear for 4 days, when it was discarded. Pupae, that developed from the larvae fed on contaminated medium for 24 hours and, subsequently sterilized with 1:500 solution of HgCl_2 for two minutes, were cultured after from 7 to 10 days. These were sterilized in 3 per cent Lysol, washed in 5 changes of sterile distilled water and crushed in tubes of a sterile nutrient broth. Of 65 pupae cultured after 7 days, 51 were positive for *E. amylovora*.

Fruit flies that developed from pupae were cultured as above 14 days after removal from the contaminated medium. Out of 78 flies, 56 were positive for *Erwinia amylovora*. Fifty empty puparia cultured revealed 40 with *E. amylovora*. Flies were left in the breeding medium to produce eggs, larvae, and pupae. Larvae of the second generation were found to carry organisms of *E. amylovora* in the internal organs. Pupae of this second generation were surface-sterilized and transferred into a freshly prepared apple medium. Out of 20 of these pupae cultured, 12 yielded a pathogenic form of *E. amylovora*. Newly emerged imagoes representing the flies of the second generation were now cultured. Among 36 of these flies, 19 contained *E. amylovora* in their viscera.

Thus it appears that the *Drosophila* flies may carry the organism from one generation to another when bred in the medium containing pathogenic organisms. This observation is in agreement with that of Leach (4, 5) and Johnson (3) who found that the bacterial flora of insects is to a large extent determined by the substrate on which it subsists. Due to the small size of the *Drosophila* eggs, it was not feasible to establish whether they carried the bacteria.

An experiment was performed to see how long the organisms of *Erwinia amylovora* are able to remain alive in the digestive organs of *Drosophila*

flies. Two hundred adult flies were fed on contaminated apple medium for 24 hours after which time they were transferred to another bottle that contained no food. The cotton plug of the bottle was wrapped with a blotting paper wetted with 1:500 HgCl_2 solution and a strip of the same kind of paper treated in the same way was allowed to extend about 2 inches into the test tube. This last procedure was undertaken in an attempt to disinfect the feet of the flies and thus reduce the chance of food contamination on their subsequent feeding. The flies were fed every 6-10 hours by removing the cotton plug and inserting a cork in the center of which was placed some apple medium. Methods of surface sterilization and culturing of flies of this experiment remained the same as those described earlier. The results are found in table 1. This table indicates that the fire-blight organisms may remain viable in the digestive organs of adult *Drosophila melanogaster* flies for as long as 6 days.

The faeces of *Drosophila* flies were likewise examined for *Erwinia amylovora* in the following manner. Before surface sterilizing, flies were introduced into a clean sterile test tube and allowed to defecate on the wall of the test tube. After this they were etherized and removed. Now, the faecal spots were touched with a sterile cotton swab moistened in sterile broth, dropped into broth which then was incubated at 28° C. Growth obtained was diagnosed for the fire-blight bacteria by inoculating shoots of *Pyrocantia angustifolia*. There was observed to exist a remarkable cor-

TABLE 1.—Persistence of *Erwinia amylovora* in viscera of *Drosophila melanogaster*, *Musca domestica*, and *Lucilia sericata*

Time interval		Hours					Days			
		0	17	24	48	72	4	5	6	9
<i>Drosophila melanogaster</i>	No. cultured ...	20	15	20	22	25	18	20	20
	No. positive	20	13	12	17	20	11	8	0
	Faeces	+	+	+	+	+	+	-
<i>Musca domestica</i>	No. cultured ...	10	12	15	14	12	-	-	-
	No. positive	10	12	10	8	0	-	-	-
	Faeces	+	+	+	-	-	-	-
<i>Lucilia sericata</i>	No. cultured ...	10	10	13	18	18	11	-	25
	No. positive	10	10	11	15	10	0	-	0
	Faeces	+	+	+	+	-	-	-

relation between the persistence of *E. amylovora* in viscera and the presence of it in defecated material (Table 1).

The common house-fly, *Musca domestica*, also was investigated in reference to the fire-blight organism. Sterilized horse dung to which a little commercial casein was added proved to be a satisfactory substratum.

In one experiment with *Musca domestica*, flies were allowed to feed on 10 per cent sucrose solution contaminated with *Erwinia amylovora* and then to deposit their eggs on the horse dung-casein mixture. These eggs were divided into two groups: one group was cultured in broth without any surface sterilization and another group was surface sterilized with 1:1000 solution of HgCl_2 for 2 minutes before culturing. Invariably eggs without sterilization carried *E. amylovora*, while sterilized eggs were always negative for this organism. Eggs deposited by *M. domestica* flies fed on contaminated sugar solution, produced larvae, pupae, and imagoes that harbored the pathogenic bacilli (*E. amylovora*).

In another experiment house flies were fed on sterile 10 per cent sucrose solution and allowed to deposit eggs in the horse dung-casein mixture. Larvae produced from these eggs were transferred to horse dung-casein mixture plus *Erwinia amylovora*. Forty-eight hours later 17 larvae were cultured after surface sterilization and were all found to harbor the bacilli of the fire-blight disease. Twelve pupae cultured from this lot were all contaminated internally with *E. amylovora*. Flies from similar pupae were cultured immediately after emergence in 2 different ways: first, they were surface sterilized with a 1:500 solution of HgCl_2 for 5 minutes and then crushed in sterile broth; second, they were thrust without sterilization into the nutrient broth, shaken for 3 minutes, and then discarded altogether. Thirty flies treated by the first method yielded 29 flies positive for *E. amylovora* and 24 flies treated by the second method contained 18 flies positive for *E. amylovora*. Forty empty puparia cultured in nutrient broth were 100 per cent positive for *E. amylovora*.

Seventy-five house-flies were fed on contaminated 10 per cent sugar solution for 24 hours and subsequently transferred into a new cage with food removed altogether but water supplied. These flies were cultured in much the same way as was *Drosophila*. Data on this experiment are included in table 1. It is evident that *Erwinia amylovora* remains in the digestive organs of the housefly for three days under laboratory conditions. Excrements of the housefly that had fed on contaminated food were found to contain viable organisms of *E. amylovora* soon after feeding and up to the fourth day thereafter.

To carry the investigation further green bottle flies, *Lucilia seriata*, were procured from the Department of Entomology of the University of California. Attempts to rear them in a manner similar to that used for *Musca*

domestica were unsuccessful. The experiment was therefore limited to adult flies. The flies were fed on contaminated 10 per cent sugar solution for 24 hours and subsequently transferred into a clean cage with sterile 10 per cent sucrose solution. Some flies were cultured immediately and some after various intervals of time. The results are found in table 1. Flies of *L. seriata* pass the organism of the fire blight through their alimentary canal uninjured and the faeces remain infective up to the fourth day, under laboratory conditions.

The honeybee, *Apis mellifica*, has been mentioned more often than any other insect in connection with the fire-blight disease. The writers aimed to study this insect in reference to the possible fate of the fire-blight organism when this organism is ingested by the bee. A large number of bees was collected in the field and confined to a cage. Honeybees do not adjust rapidly to confinement and a large number of them die soon after capture. Bees were fed on 10 per cent contaminated (*Erwinia amylovora*) sucrose solution for 24 hours and after that only on sterile sugar solution. This solution was changed every 2 hours to minimize the danger of recontamination with the organism. After 24 hours feeding, 5 bees were surface-sterilized in 5 per cent Lysol solution for 5 minutes, washed in 6 changes of sterile distilled water and cultured in broth by crushing with a sterile needle. All 5 yielded virulent cultures of the fire-blight organism. Now, the bees after surface sterilization were cultured in the following manner: the head and the thorax were pulled out with the flamed forceps while the abdomen was held with another pair of sterile forceps. In this way it was possible to separate the nectar stomach from the abdomen. The findings are tabulated in table 2. The data presented show that the fire-blight organism may remain viable in viscera and nectar stomachs of the honeybees for as long as 48 hours under controlled laboratory conditions.

TABLE 2.—Persistence of *Erwinia amylovora* in nectar stomachs and other viscera^a of the honeybee

Time interval	Number of cultured:		Cultures positive for <i>E. amylovora</i>	
	Viscera	Nectar stomachs	Viscera	Nectar stomachs
24 hours after feeding	10	10	10	10
48 " " "	10	10	10	8
72 " " "	20	20	10	7
96 " " "	10	10	0	0
120 " " "	10	10	0	0

^a By other viscera the writers mean abdomen when head, thorax, and nectar stomach are removed.

To ascertain whether *Erwinia amylovora* is able to stay alive in the mouth parts of the honeybee some 50 bees previously fed on contaminated sugar solution were cultured soon after feeding and 24 hours after feeding. In each case heads were surface-sterilized. No bacteria were isolated. Heads of another lot of 50 bees were cultured without any surface sterilization to learn how long the organism of *E. amylovora* may remain on it. These bees were fed on contaminated sugar solution and in addition sprayed with it to be sure of the presence of the organism on the heads. Ten bees cultured after each of the intervals indicated gave 10 positive when cultured immediately, 6 positive after 6 hours, 7 positive after 10 hours, 4 positive after 12 hours and all negative after 24 hours. This finding is in essential agreement with that reported recently by Pierstorff and Lamb (7). Attempts to isolate the organisms from faeces of bees were unsuccessful in a number of tests. The data show that the honeybees may ingest the pathogen with contaminated food, harbor it in their nectar stomachs and other viscera but destroy it, apparently by secretions in a relatively short time. These results offer a possible explanation of the lack of infection when known infested beehives are confined with blossoming trees (6, 7, 13).

From the point of view of epiphytology, the knowledge of transmission of disease by insects is of paramount importance, be it external or internal transmission. Obviously enough, the internal transmission and especially biologic internal transmission of a pathogen deserves especial consideration, since in this case the insect may retain the organism over a shorter or longer period unfavorable for development in the susceptible plant and thus the severity and duration of an epiphytotic may be greatly accentuated.

The experiments reported above seem to demonstrate that the dissemination of the fire-blight organism takes place by biologic transmission in addition to the known mechanical transmission.

SUMMARY

Larvae of *Drosophila melanogaster* and *Musca domestica* after feeding on a medium contaminated by *Erwinia amylovora* were found to contain the organism in their internal organs. The bacteria persisted through the pupa to the adult in both of these insects.

Eggs of *Musca domestica* from contaminated females carried the organism externally but not internally.

Adults of *Drosophila melanogaster*, *Musca domestica* and *Lucilia seriata* harbored the organism internally for 6, 3, and 4 days, respectively.

The bacteria remained viable in the viscera of honeybees for 48 hours but were not recovered from the heads after 12 hours from the time of contamination.

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RESISTANCE TO CLADOSPORIUM FULVUM¹

E. F. GUBA

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The study of resistance in the genus *Lycopersicum* to *Cladosporium fulvum* Cke. and the development of tomatoes resistant to leaf mold is now receiving recognition in several countries. An account of the progress made and the possibilities that the work so far indicates may be of interest to plant pathologists at this time. That the control of this disease, which has wrought havoc to the greenhouse tomato industry, may be accomplished through the science of genetics now seems probable.

Cladosporium fulvum is narrowly confined to the tomato, *Lycopersicum esculentum* Mill. (20), (35), (15, 17), (7, 8), (31). The cherry and plum tomatoes (f. *cerasiforme* Alef. and f. *pruniforme* Alef.) are susceptible, but the red currant tomato, *L. pimpinellifolium* Mill. is completely resistant (31), (17), (7, 8). In the writer's studies the potato-leaved (f. *grandifolium* Bailey), as represented by the variety Wayahead, the pear (f. *pyriforme* Alef.), and the plum tomatoes were very susceptible, and yellow cherry was more resistant than red cherry, although both were quite susceptible. In the closely related genera *Physalis* and *Solanum*, all the members tested proved resistant, but the writer's attempts to hybridize members of these genera with the cultivated tomato failed.

Certain varieties of the cultivated tomato (f. *vulgare* Bailey) have been reported to possess some resistance to leaf mold. This resistance is expressed by less growth of mycelium and less sporulation than occur on susceptible varieties and by the production of yellow blotches centering around the point of infection with but a scant growth of fungus. The foliage is slower to become infected, at least the incubation period appears to be longer, and the vines remain green longer than those of susceptible varieties, although under continued favorable conditions for infection, which are frequent in the fall greenhouse cropping season, the foliage of these somewhat resistant varieties ultimately becomes badly diseased. Therefore, under commercial growing conditions, the disease may affect these varieties seriously enough to be a limiting factor in the production of fruit.

Reported observations regarding the resistance of varieties of the common cultivated tomato to leaf mold are not consistent (Table 1). This appears to be due to variability in the growing conditions, where small differences influence the degree of infection, rather than to the existence of different strains of the fungus. In the sense of possessing the greatest

¹ Contribution No. 216 of the Massachusetts Agricultural Experiment Station.

TABLE 1.—*Varieties of tomato reported most resistant to Cladosporium fulvum*

Variety	Alexander (7)	Bewley (9)	Boock (10)	Guba (15), (16)	Heydemann (21)	Jagger (22)	Makenson (23)	Newhall (24)	Norton (25)	Quinn (27)	Rath (29)	Sengbusch and (31) Loschakowa-Hasenbusch	Small (33, 34)	Springer (36)
Frogmore Selected				-						-			+	
Lucullus	-		+	+							+			
Maincrop	+			+				+				-	+	
Norduke	+			+		+							+	
Satisfaction	+			-				+					+	
Sterling Castle	+	+	+	-		+			+	-		+	+	
Stone	-			-			-		+			-	+	
Tuckswood	-		-	-	+						+	-		+
Up-to-Date	+	+		+	+								+	
Riverside × Up-to-Date (Bewley)...				+										
Stonnor's M.P. × Up-to-Date (Bewley)				+										
Maincrop × Up-to-Date (Bewley)				+										
ES 1 × Up-to-Date (Bewley)				+										
Norton	-			+										

+ Indicates resistant

- Indicates not resistant

measure of resistance and of showing value for developing hybrids somewhat resistant to leaf mold, the varieties Maincrop, Norduke, Up-to-Date, Norton, and certain hybrids originated by Bewley², appeared promising in the writer's studies, while Frogmore Selected, Lucullus, Stone, and Tuckswood, which have been reported to show resistance, proved very susceptible. Sterling Castle and Satisfaction were somewhat resistant, but also too susceptible to be considered of positive value in hybridization work.

Some reports of progress have appeared relating to the hybridization of tomatoes for resistance to leaf mold (9), (24), (16, 17), (31), (4, 5, 7). In these investigations, the varieties Maincrop, Up-to-Date, Sterling Castle, Satisfaction, and Norduke have rather consistently been found to offer most promise as a source of resistance for hybridization purposes (Table 2). The resistance of these varieties, as is shown in crosses of Sterling Castle, rests on recessive factors (31), (7). Since the progeny of these crosses

² In 1931 the writer received from Dr. W. F. Bewley, Director of the Experimental and Research Station, Cheshunt, England, seed of F₄ selections of hybrids of Up-to-Date with Riverside, Stonnor's M.P., Maincrop, and ES 1.

TABLE 2.—Crosses of standard varieties of tomato developed for resistance to *Cladosporium* leaf mold.

Alexander (4, 5, 7)	Newhall (24)
Sterling Castle × Marhio	Satisfaction × Bonny Best
Sterling Castle × Globe	Satisfaction × Marhio
Satisfaction × Marhio	Maincrop × Bonny Best
Satisfaction × Globe	Maincrop × Marhio
	Sengbusch and Loschakowa-Hasenbusch (31)
	Sterling Castle × Susceptible varieties
Bewley (9)	Guba (16, 17, 18)
Up-to-Date × ES 1	Up-to-Date × Norduke
Sterling Castle × ES 1	(Up-to-Date × Riverside) × Norduke
Up-to-Date × Riverside	(Up-to-Date × Stonnor's M.P.) × Norduke
Up-to-Date × Tuckswood	(Up-to-Date × Maincrop) × Norduke
Up-to-Date × Maincrop	(Up-to-Date × ES 1) × Norduke
Up-to-Date × Manx Marvel	Maincrop × Norduke
Up-to-Date × Stonnor's M.P.	

have seriously succumbed to infection under conditions favoring epidemics of leaf mold, more recent reports show that these efforts are being abandoned (7), (18). In the work of the writer even the reaction of the progeny of crosses of the somewhat resistant English types, such as Maincrop, Up-to-Date, and the Bewley hybrids with the somewhat resistant large-fruited American variety Norduke, was disappointing under the usual greenhouse conditions favorable for the disease.

Efforts to develop a desirable greenhouse tomato resistant to leaf mold have been confined largely and more recently to the hybridization of *L. pimpinellifolium* with *L. esculentum* f. *vulgare* (31), (17, 18), (6, 7, 8), and Wenholz.^{3,4} It appears fortunate that this work is being done in different parts of the world, for it is believed that the objective of developing desirable greenhouse types of tomatoes for each particular market will be more readily achieved by the efforts of several investigators. Immunity has been shown to be expressed by a single dominant Mendelian factor. Excellent studies of other inheritance characters resulting from the hybridization of red currant with standard tomatoes have been reported by Groth (11, 12, 13, 14), Price and Drinkard (26), and others.

³ In a letter to the writer dated May 13, 1935, it was learned that Dr. H. Wenholz of the Department of Agriculture, New South Wales, has also engaged in the hybridization of red currant with standard tomatoes for developing resistance to fusarium wilt. Some lines of his many crosses carried as far as the 6th generation for size of fruit and resistance to wilt were sent to the writer for study of their reaction to *cladosporium* leaf mold. In this study none showed resistance to *cladosporium*.

⁴ The extensive work of Agerberg *et al.* (1, 2, 3) and Schmidt (30) to explain the high resistance of *L. pimpinellifolium* Mill. to *Cladosporium fulvum* Cke. unfortunately is referable to *Trichothecium roseum* (Pers.) Lk., a saprophyte (Raabe and Sengbusch (28)). It is hoped that further work at Müncheberg, Germany, will correct the disappointment that has resulted from this error.

It now appears definitely probable that large-fruit tomatoes, completely resistant to the disease, will be developed, which should be of great value to the greenhouse-tomato industry. With the purpose solely of developing an early-fruited tomato for North Dakota, Yeager (37) hybridized the red currant with Bison and subsequently crossed selections of this hybrid of determinate growth habit with Pink Heart for size. The manifestation of earliness and unusual flavor offered by crosses of the red currant with common standard varieties (32), (19) would seem to have far reaching value aside from the matter of resistance to cladosporium leaf mold if the inference is true that such tomatoes may serve in the human diet as substitutes for orchard fruits.

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION,
DEPARTMENT OF BOTANY,
FIELD STATION, WALTHAM, MASSACHUSETTS.

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PHYTOPATHOLOGICAL NOTES

A Method for the Isolation of Actinomycetes from Scab Lesions on Potato Tubers and Beet Roots.—Many taxonomists consider the Actinomycetaceae and Mycobacteriaceae as families of the order Actinomycetales. A mixture of sodium hypochlorite and sodium hydroxide is used in the isolation of certain members of the second family.¹ It was suggested by W. H. Burkholder that such a mixture might be of value in the isolation of plant pathogenic actinomycetes. Since calcium hypochlorite is a common laboratory disinfectant this was substituted for the sodium salt.

Preliminary trials were conducted and the following technique was found satisfactory. Calcium hypochlorite is prepared as described by Wilson.² Ten grams of fresh calcium hypochlorite is thoroughly shaken in 140 cc. of tap water. This is allowed to stand a few minutes and then filtered. Immediately before using, 1 part of sodium hydroxide solution (250 grams of sodium hydroxide dissolved in water and made up to 1000 cc.) is added to 3 parts of the above filtrate.

Tubers are placed in the above mixture for 2 minutes, removed, and, without washing, a slice is cut to remove a lesion and the underlying healthy tissue, using a sterilized scalpel. This slice is placed in a few drops of sterilized distilled water in a flamed mortar and thoroughly triturated with a flamed pestle. After triturating the tissue additional water is added to the mortar and the diluted suspension transferred to a sterilized tube by pipetting. The use of a tube of sterilized water with a pipette inserted through the plug greatly facilitates this procedure.

After a number of tubes of the suspension have been prepared 1 or 2 drops from a tube are placed in the bottom of a sterilized Petri dish. About 15 cc. of Waksman's³ egg-albumin agar,⁴ which has been melted and cooled to 45° C., is then poured into the dish. The dish is carefully rotated to distribute the suspension through the medium. Colonies develop in from 2 days to 3 weeks, depending on the strain present and the incubation temperature.

¹ Park, W. H., A. W. Williams and C. Krumwiede. The bacillus and bacteriology of tuberculosis. p. 492-526. In *Pathogenic Microorganisms*. 9th ed. 819 pp. Lea and Febiger, Philadelphia. 1929.

² Wilson, J. K. Calcium hypochlorite as a seed sterilizer. *Amer. Jour. Bot.* 2: 420-427. 1915.

³ Waksman, S. A. Cultural studies of species of Actinomyces. *Soil Sci.* 8: 71-207. 1919.

⁴ "Egg-albumin agar. Dextrose, 10 gm.; K_2HPO_4 , 0.5 gm.; $MgSO_4$, 0.2 gm.; $Fe_2(SO_4)_3$, trace; egg albumin, 0.15 gm.; agar, 15 gm.; distilled water, 1000 cc. The egg albumin is dissolved in N/10 NaOH until neutral to phenolphthalein, then added to the warm medium."

The writer has used this method since the spring of 1930 with comparatively little trouble from contamination by other microorganisms. This disinfection method suppresses bacterial contaminants. When certain other media, as potato-dextrose or beef-extract agar, are used instead of Waksman's agar, fungal contaminants may overrun the dish before the *Actinomyces* colonies develop sufficiently for transferring. No difficulty has been experienced in isolating *Actinomyces* from scabbed tubers at any time after the lesions become evident.—C. F. TAYLOR, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

*Physalis subglabrata: A Natural Host of Bacterium angulatum.*¹—All attempts to control the two bacterial diseases of tobacco, angular leaf spot and wild fire, based on the assumption that tobacco is the only host, have failed. In spite of all precautions to prevent infection from previous tobacco crops, leaves protruding through a tear in the cotton covering of the bed may develop a few wild-fire or angular leaf spots with no evidence whatever of any other infection. Closely boxed beds are usually freer from infection than nonboxed beds. Perfectly clean plants may be set in the field only to develop one or the other or both diseases following a rainy period.

The writers have believed that infection was carried over winter in weeds, but have previously failed to obtain evidence, other than observational, in favor of this hypothesis. Inoculation studies of J. Johnson *et al.*² favored the weed-host theory. They claimed a wide host range for *B. tabacum*. Clayton³ showed, however, that the bacterial toxin present in broth used as inoculum was responsible, in the species studied, for the development of wild-fire spots. He was unable to demonstrate extensive multiplication of the organism except in species of *Nicotiana*, and concluded that only species of *Nicotiana* should be regarded as hosts of *Bacterium tabacum*. Beach⁴ observed ground cherry plants (*Physalis virginiana*) with typical wild-fire lesions, growing near affected tobacco. The spots centered on injuries made by flea beetles. He isolated *B. tabacum* from these spots and believed that he had evidence that the parasite had multiplied within the leaf. He concluded, however, that "the results obtained with *Bacterium tabacum* upon *Physalis virginiana* seem to correspond in most respects to those reported by Clayton for a series of garden plants not belonging to the

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

² Johnson, J., C. M. Slagg and H. F. Murwin. Host plants of *Bacterium tabacum*. *Phytopath.* 14: 175-180. 1924.

³ Clayton, E. E. Toxin produced by *Bacterium tabacum* and its relation to host range. *Jour. Agr. Research* [U. S.] 48: 411-426. 1934.

⁴ Beach, W. S. Control of tobacco wildfire (second report). *Penn. Agr. Expt. Sta. Bul.* 322, 1935.

genus *Nicotiana*." After reading Beach's report, the present writers concluded, on the contrary, that the key to the solution of the sudden appearance of wild fire had probably been found. Consequently, plants of smooth ground cherry *Physalis subglabrata* M. & B. growing in pastures and fence-rows at a distance from tobacco on the Experiment Station farm were immediately examined and found to be extensively spotted. Bits of tissue from the spots, examined microscopically, were found to be teeming with bacteria having motility typical of a polar flagellate organism. Bits of the spots dropped into broth used as inoculum on tobacco plants produced typical angular leaf spot. From 37 of 39 plants of *Physalis subglabrata* and from the only plant of clammy ground cherry worked with, typical angular leaf spot was obtained on tobacco plants, from which pure cultures of *B. angulatum* were obtained at will. A single colony from a typical angular leaf spot on tobacco was found to contain two strains of *B. tabacum*, a smooth, and a somewhat rough concentric colony strain, each of which caused typical wild-fire spots on tobacco.

The first collections were made October 31, two months after tobacco had been cut. Most of the inoculation studies were made after the groundcherry tissue had dried. As the weeds were growing at a distance from tobacco plants there was no opportunity for them to have become infected by drip from diseased tobacco plants. If the bacteria originated in tobacco, they must have been carried in relatively small numbers to the groundcherry where they became established and multiplied. The affected groundcherry leaves had all been gnawed by flea beetles. Beach believed that flea beetles were unable to transmit wild fire, although Wolf⁵ had demonstrated in 1922 that they were able to do so. The writers found in the spring of 1935 that *Bacterium angulatum* could be isolated from the bodies of flea beetles and garden flea hoppers. Flea beetles feed upon physalis in the spring of the year and later migrate to tobacco beds, and in large numbers to fields recently set with tobacco. Injury to recently set plants is often extensive. *Bacterium tabacum* and *B. angulatum* both live through the winter in tobacco trash left out-of-doors, so there is no reason for believing that they will not live in affected physalis leaves also. Reinfection of *Physalis* could readily occur in the spring, and migration of flea beetles from it to tobacco could account for a part, at least, of plant-bed and field infection.

The evidence obtained up to the present seems sufficient to prove that *Physalis subglabrata* is a natural host of *Bacterium angulatum* and that plants of this species growing at some distance from tobacco go into the winter heavily infected. Sudden outbreaks, and equally sudden subsidence, of wild fire in a region may depend upon the extent to which *Physalis* becomes infected in the fall. Future work on the epiphytology of angular leaf spot and wild fire must take *Physalis subglabrata* and perhaps other

⁵ Wolf, F. A. Wildfire of tobacco. N. C. Agr. Expt. Sta. Bul. 246. 1922.

solanaceous weeds into consideration.—W. D. VALLEAU AND E. M. JOHNSON, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

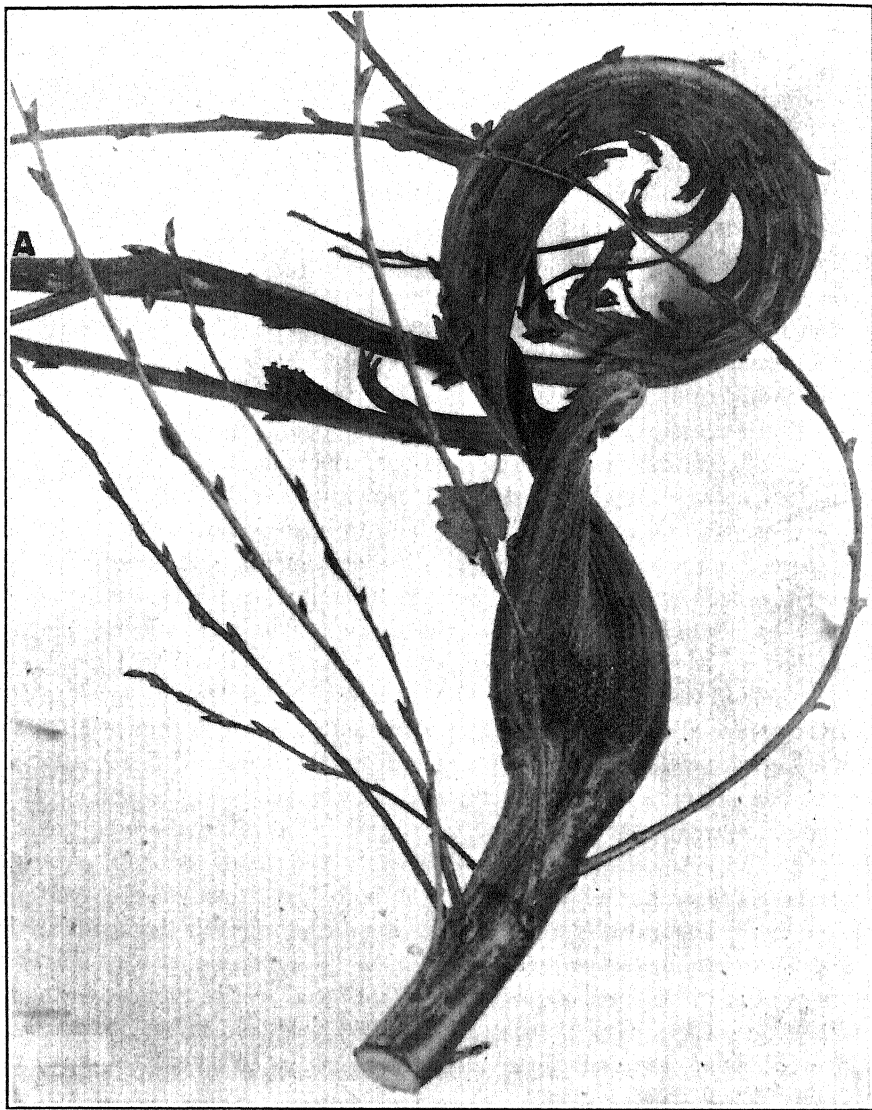


FIG. 1. Fasciated leader of European birch. A indicates position of twigs shown in figure 2. Approx. $\times 0.4$.

Fasciation of Betula pendula dalecarlica.—On October 3, 1935, a striking case of fasciation was found on the tip of the main stem of a 25-foot

European birch, *Betula pendula dalecarlica* Schneid., in Washington, D. C. The rest of the tree appeared to be perfectly normal.



FIG. 2. Non-separated buds and twigs of fasciated European birch at end of branch A of figure 1. Approx. $\times 1$.

The flattened and twisted development of the leader is shown in figure 1. Near its extreme tip (Fig. 2) is a cluster of buds that failed to separate. The contour of the twigs and their partial separation below the tip clearly indicate that the flat band-like expansion is at least partly caused by the failure of the twigs to separate and is not the abnormal development of a single branch.

Although fasciation is very common on some plants, in the literature there are comparatively few references to it on deciduous trees. The failure to find any reference to fasciation on birch leads one to conclude that the disease is rare and of no economic importance.—MARVIN E. FOWLER, Division

of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

Powdery Mildew of Potato in New Jersey.—During February, 1935, a leaf-spot disease appeared on some potato plants of the variety Green Mountain growing in the greenhouse at The Rockefeller Institute for Medical Research, Princeton, New Jersey. The spots began as small, irregularly shaped areas not more than 2 mm. in diameter. They were most numerous on the upper surfaces of leaves that were nearing maturity. As the spots enlarged, their color darkened. Confluent spots sometimes involved large areas of tissue. The disease persisted on potted plants in greenhouses during the past summer, but was not observed in nearby potato fields.

In periods of cloudy weather the fluffy white mycelium of a fungus developed over affected areas (Fig. 1). The fungus appeared in spots on both the upper and lower surfaces of leaves, on leaf petioles, and on stems. Microscopic examination of fresh leaf tissues and of stained sections of leaves showed it to be a powdery mildew. Numerous barrel-shape conidia borne in chains on short conidiophores were observed. When germinated in water, the conidia produced short, straight germ tubes. The mycelium was found to be superficial and attached to epidermal cells by means of globoid haustoria. As no perithecia were produced, it has not been possible to determine the species to which the fungus belongs, but it is presumed to be *Erysiphe solani* mentioned by Vaňha in 1902.¹ The conidia were found to have an average length of $34.19 \pm 3.08 \mu$ and an average width of $18.09 \pm 1.61 \mu$, as determined by measurements on 100 mature spores taken at random from leaf spots. These average dimensions are in fairly close agreement with those reported by Müller² for the conidia of a mildew occurring on potatoes in Germany. Potato mildew has been recorded from France,^{3, 4} Russia,⁵ and Peru,⁶ but, so far as the writer is aware, it has not been reported previ-

¹ Vaňha, J. Eine neue Blattkrankheit der Rübe. Ztschr. Zuckerindus. Böhmen 27: 180-187. 1902-03.

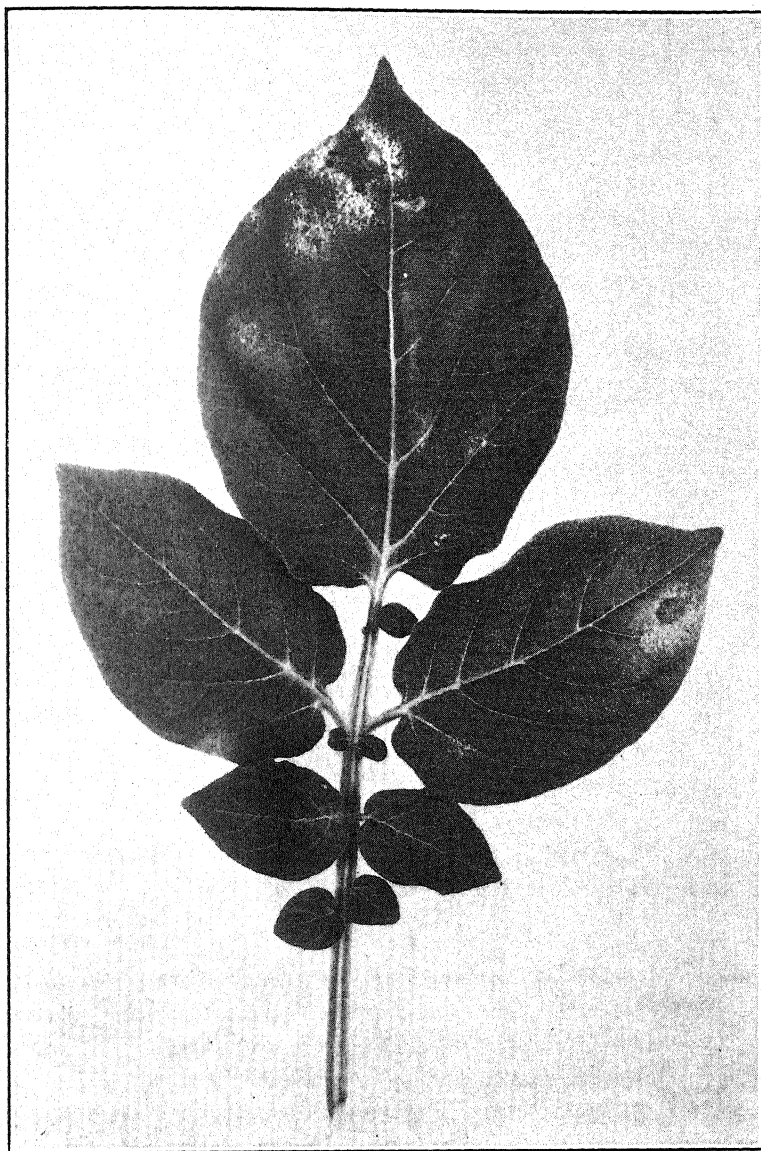
² Müller, K. O. Ueber den "echten Mehltau" der Kartoffel. Nachrichtenbl. Deutsch. Pflanzenschutzd. 8: 19-20. 1928.

³ Ducomet, V. Oïdium de la pomme de terre et Oïdium de la betterave. Soc. Path. Vég. France Bull. 8: 153-154. 1921.

⁴ Marchal, P., and E. Foëx. Rapport phytopathologique pour les années 1926-1927. Ann. des Épiphyties [France] 13: 383-454. 1927.

⁵ Jaczewski, A. A. Parniatnaia Knizhka ialia zapisei dannikh fitopatologichkogo osmotra Kartofelnykhs nasazhdenii. [Notebook for entering data obtained from the phytopathological survey of potato crops.] A. A. Jaczewski Mycological Laboratory, Leningrad. 1926. Review in Rev. Appl. Mycol. 6: 250. 1927. In Russian. (Original article not seen.)

⁶ Abbott, E. V. Further notes on plant diseases in Peru. Phytopath. 21: 1061-1071. 1931.



Photograph by J. A. Carlile.

FIG. 1. Potato leaf affected by powdery mildew.

ously from the United States.—L. O. KUNKEL, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Elsinoe fawcetti, the Perfect Stage of the Citrus Scab Fungus.—A myriangiaceous ascomycete, typical of the genus *Elsinoe* and interpreted as

the perfect stage of the citrus-scab organism, was found recently on scab lesions on ripe rind of Satsuma orange, *Citrus nobilis unshiu* Sw. (Fig. 1-A). The specimen, consisting also of scabbed green rind and leaves of Satsuma, was collected on May 28, 1935, at M'Boy, São Paulo, by A. O. Martins, in connection with routine inspection of citrus in this locality, and sent to the Phytopathological Section of the Instituto Biologico de São Paulo. It was retained particularly for the purpose of obtaining a culture record of *Sphaceloma fawcetti* from this variety of orange. The isolation (No. 228C, Phyt. Sect. Inst. Biol. de São Paulo) was made on May 31, the day the specimen was received, by means of platings from rind lesions.

Dark punctiform fungus masses on the lesions (Fig. 1-B and C) were sectioned on Aug. 21, 1935, and these proved to be the ascomata of an *Elsinoe* (Fig. 1-D to I). An attempt was made to establish the relationship of the newly found fungus with *Sphaceloma fawcetti* by means of cultures from ascospores (Fig. 1-J); but, unfortunately, they produced no growth. Nevertheless, it is believed that this ascomycete is unquestionably the perfect stage of *S. fawcetti*. In the first place, the specimen is typical of citrus scab and the conidial stage of *S. fawcetti* is present on the younger lesions of the specimen, *i.e.*, the leaf lesions. Moreover, the culture isolated from scrapings of the rind lesions is typical of this fungus. Secondly, the occurrence of the *Elsinoe* on the scabs and its close resemblance to other species of the same genus, for example, *E. ampelina* (de By.) Shear, *E. piri* (Wor.) Jenkins, *E. phaseoli* Jenkins, that have been shown to be the perfect stage of other species of *Sphaceloma*, is sufficient indication of the genetic relationship between the newly discovered ascomycete and the citrus-scab organism. Since the *Elsinoe* has not been described heretofore, the name *Elsinoe fawcetti* is proposed.

***Elsinoe fawcetti* n. sp.**

Ascomata more or less scattered, pulvinate, dark brown, circular to elliptical, $38-106 \times 36-80 \mu$; epithecium composed of dark colored pseudoparenchyma, $5-9 \mu$ thick; asci 1 to 20 or more in a single ascoma, distributed in the lighter colored stromatic region beneath the epithecium, globose to ovoid, $12-16 \mu$ diameter, wall of unexpanded ascus thickened in upper portion; ascospores hyaline, oblong-elliptical, $10-12 \times 5-6 \mu$; 2-4 celled, usually constricted at the middle septum, upper half of spore thicker and shorter, lower half thinner and longer; epispore 1.2μ . Conidial stage, *Sphaceloma fawcetti* n. sp. (p. 101).¹

Ascomata plus minusve sparsa, pulvinata, atro-brunnea, orbicularia vel elliptica, $38-106 \times 36-80 \mu$; epithecium obscuro-pseudoparenchymaticum, $5-9 \mu$ crassum; asci 1-20 vel plures in ascomate singulo, sub epithecio in

¹ Jenkins, A. E. The citrus scab fungus. Phytopath. 15: 99-104. 1925.

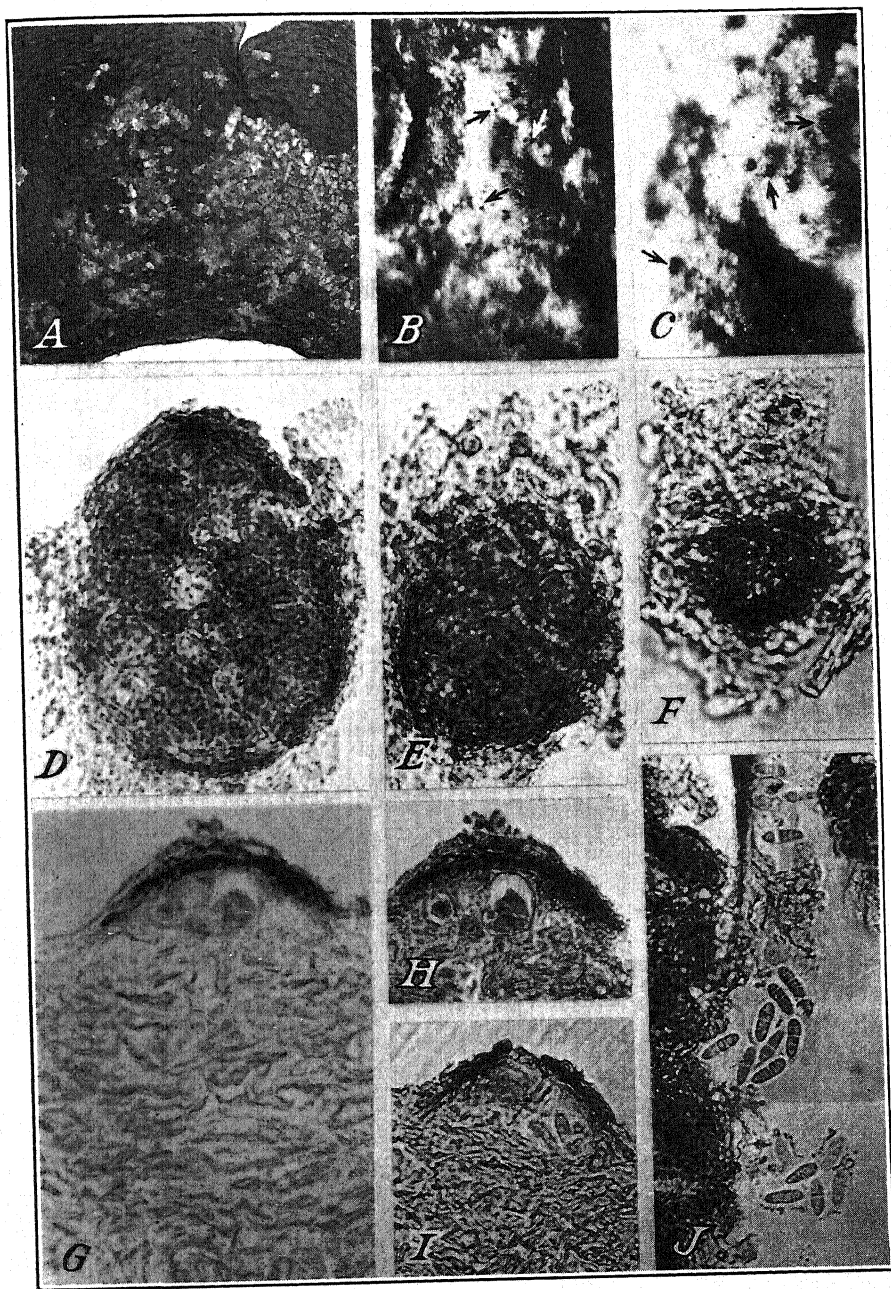


FIG. 1. *Elsinoe fawcetti* on rind lesions of Satsuma. A. Part of type ($\times 1$). B. and C. Ascomata on scabs (B $\times 22$; C $\times 60$). D-E. Ascomata; circular outline of asci indistinctly visible through epithecium. F. Ascoma with single ascus. G-I. Sections of scab with ascoma. H. Same as G, but stained with cotton blue. J. Ascospores. (D-J $\times 500$).

regione stromatica pallidiore dispositi, globosi vel ovoidei, 12–16 μ in diam.; asci inexpandi superne crasse tunicati; ascospori hyalini, oblongo-elliptici, 10–12 \times 5–6 μ , 2–4-cellulati, plerumque ad septum medium constricti, parte superiore crassiore et brevior, parte inferiore tenuiore et longior; epispodium 1.2 μ crassum.

On *Citrus nobilis unshiu* Sw., Brazil, São Paulo (Bairro de M'Boy) Chacara de Sumakiti Morita, May 28, 1935, A. O. Martins. In Herb. Phytopath., Instituto Biologico de São Paulo, Brasil, No. 2041 (Type of perfect stage). Part of specimen in Mycol. Coll. Bur. Plant Industry, U. S. Dept. Agric., Washington, D. C.—A. A. BITANCOURT, Instituto Biologico de São Paulo, Brazil, and ANNA E. JENKINS, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Reaction of the Victoria Oat Variety to Crown Rust.—The high resistance of the Victoria oat variety (C. I.¹ No. 2401) to crown rust (*Puccinia coronata avenae* Eriks. and Henn.) in North America was first reported by Murphy and Stanton.² This unusual resistance to crown rust, as well as high resistance to loose smut (*Ustilago avenae* (Pers.) Jens.), and covered smut (*U. levis* (Kell. and Sw.) Magn.), has resulted in the rather wide use of Victoria by oat breeders as a source of resistance to these diseases.

Unfortunately, some of the Victoria stocks do not react homozygously to crown rust. Numerous pure-line selections have been obtained from the variety and tested with different physiologic forms of the rust. While all of these lines were apparently morphologically identical, a few were completely susceptible to various forms of crown rust. However, a high percentage of the lines were resistant to all the forms to which they were subjected. The presence of one of these susceptible lines already has been reported by the writer,³ but its practical significance was not emphasized. There is evidence that Victoria embraces also some smut-susceptible lines. Their presence in the original mass stock of Victoria has negated the value of several early and important crosses made by the writer and his coworkers in that these susceptible lines happened to be used as parents. Therefore, it seems desirable to report the fact and emphasize the importance of using as parent material only those pure-line selections of the original Victoria that have been definitely tested for their reactions to crown rust and smut.

Stanton and Murphy⁴ previously have stated the lot of seed accessioned

¹ C. I. refers to accession number of the U. S. Dept. Agr., Bur. Plant Indus., Division of Cereal Crops and Diseases.

² Murphy, H. C., and T. R. Stanton. Oat varieties highly resistant to crown rust. Jour. Amer. Soc. Agron. 22: 573–574. 1930.

³ Murphy, H. C. Effect of crown rust infection on yield and water requirement of oats. Jour. Agr. Research [U.S.] 50: 387–411. 1935.

⁴ Stanton, T. R., and H. C. Murphy. Oat varieties highly resistant to crown rust and their probable agronomic value. Jour. Amer. Soc. Agron. 25: 674–683. 1933.

under C. I. No. 2401 represents an artificial population of the selections 64q, 64r, and 64t. These original selections were tested by the writer and found not to be pure within themselves in so far as concerned their reaction to forms 1, 3, 7, 16, 30, and 33 of crown rust. Pure lines were obtained from each of these original selections, which were completely susceptible to one or more of the above-mentioned forms. Although like Victoria, nearly all these lines were resistant to all forms used, the original selections each contained a larger proportion of susceptible types than are found in Victoria. Apparently, Victoria has been purified since these original selections were massed, or these original selections have been subjected to more mixture than has the Victoria variety.

Except for the presence of these lines in Victoria that are susceptible to crown rust and possibly to smut, this variety has been resistant to 37 physiologic forms of crown rust collected in the United States, Mexico, and Canada, in the 9-year period from 1927 to 1935 and to all collections of smut used in field and greenhouse studies at Ames, Iowa, during the same period. Victoria does not show so high a type of resistance to certain forms of crown rust as does Bond (C. I. no. 2733). When grown immediately adjoining heavily rusted plots, its yield may be reduced because of severe leaf necrosis resulting from primary infection. Under field conditions, however, where the amount of infection is naturally restricted because of the partial or complete elimination of the source for secondary and subsequent infections, a strain of oats endowed with Victoria's resistance to crown rust has ample protection, even when heavy epiphytotics are present. Although Victoria is poorly adapted to the principal oat-growing regions of the United States and is further undesirable because of the presence of a heavy, twisted, geniculate awn, it is nonetheless valuable to the oat breeder as a source of resistance to crown rust and smut.—H. C. MURPHY, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station.

*A Method for Inoculating Wheat and Barley with Loose Smuts.*¹—Adequate testing of the reactions of varieties and selections of wheat and barley to loose smuts, as well as an extensive study of physiologic specialization in the smuts themselves, depends largely upon the use of a rapid and effective method of inoculation. An apparatus has been devised that subjects the wheat or barley head to a partial vacuum while completely submerged in an aqueous spore suspension. In this way as many as 30 heads per hour can be inoculated.

¹ Paper No. 1378 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

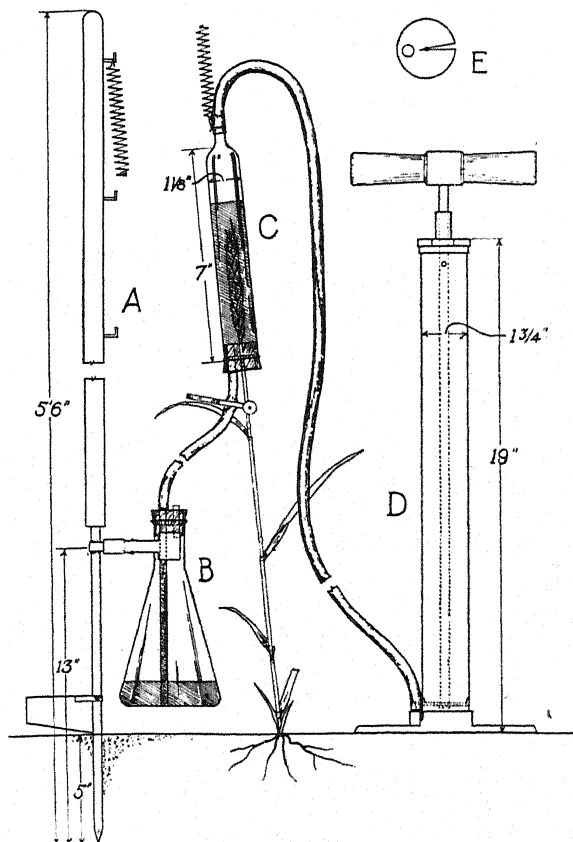


FIG. 1. Apparatus for inoculating wheat and barley with loose smuts.

The apparatus (Fig. 1) comprises a support (A); a flask for inoculum (B); an inoculating chamber (C); and a hand-operated vacuum pump (D). The lower portion of the support rod is made of 5/16 in. iron rod to which are welded an iron step to aid in pushing the rod into the ground and an old apparatus clamp to support the inoculum flask. The upper portion is best made of bamboo or light wood, equipped with a number of light, spring-wire hooks. The inoculating chamber can be made from a large test tube, or a long filter tube. The lower end (larger end) is fitted with a very soft, split rubber stopper (section at E, figure 1) and is connected through a length of rubber tube and a Mohr pinch cock with the inoculum flask. Four or five feet of thick-wall, small-bore tubing connect the top of the chamber with the vacuum pump. The inoculating chamber with stopper and tubes is hung from the support rod by a weak spring, such as may be taken from a small curtain roller. A large automobile pump with an inverted plunger leather makes a satisfactory vacuum pump. A $\frac{1}{8}$ -in. vent hole is bored

through each side of the cylinder of the pump at a point just below the upper limit of operation of the plunger, so that normal pressure is suddenly restored when the plunger is raised above it. It is important to keep the internal volume not filled by liquid as small as possible in order to create the greatest possible vacuum with each stroke of the pump.

The procedure for inoculation is simple. Fill the inoculum flask (B) with a suspension of spores, made up in the proportion of about two medium-size smutted heads in 100 cc. of water. Push the support rod (A) into the ground near the plant to be inoculated and hang the spring and inoculating chamber on one of the hooks so that a slight extension is necessary to accommodate the chamber to the height of the plant. Spread the split rubber stopper and place it around the stem just below the head; then insert head and stopper into the inoculating chamber. With barley enclose the leaf sheath and the stem, since the stem alone is too weak to survive the treatment. Open the pinch cock between B and C and draw the inoculum up into C by a gentle stroke of the pump. When the head is covered, but the chamber not more than $\frac{3}{4}$ full, close the pinch cock and operate the pump vigorously. Four to six strokes will suffice for wheat, but barley may require 10 to 15 or even more.

The partial vacuum expands the air in the florets, and the returning pressure replaces it with the liquid inoculum. Some air will leak through and around the stem, but if the pump is worked rapidly this offers no serious difficulty. When inoculation is complete, the excess inoculum may be returned to the flask by opening the pinch cock after raising the pump plunger above the vent holes. In this way the liquid may be used repeatedly, and 300 cc. will be found sufficient to inoculate 60 to 80 heads.

Results from inoculations made in 1933 and preliminary results from inoculations made in 1934 indicate that the method is highly successful for

TABLE 1.—*Loose-smut infection in 1933 resulting from vacuum inoculation of Reward wheat*

Source of inoculum		Total plants	Plants smutted	
Place	Variety		Number	Percentage
Minnesota	Marquis	22	21	95
Minnesota	Reward	21	19	90
Minnesota	Minturki	25	24	96
Oklahoma	Unknown	24	24	100
South Dakota	Marquis	25	24	96
Texas	Durum (variety unknown)	23	0	0
Iowa	Winter wheat (variety unknown)	84	0	0
Check	Uninoculated	26	0	0

loose smut of wheat and somewhat less so for loose smut of barley. The results of inoculations on Reward wheat in 1933 are given in table 1. These inoculations resulted either in no infection or in a very high degree of infection, indicating that the variation was due to differences in strains of smut used rather than to inconsistencies in the method. Eight different collections of barley loose smut were inoculated separately into 2 to 7 varieties of barley in 1933. Infection in the resulting plants ranged from 2 to 76 per cent with an average of 26.4 per cent.

Inoculation somewhat reduces the number of seeds that develop per head. For this reason, only the most vigorous heads should be selected for treatment. The most favorable stage of development, both for set of seed and for infection, seems to be just after anthesis in most of the florets and before the ovaries have more than doubled their original size.—M. B. MOORE, University Farm, St. Paul, Minnesota.

Crown Gall on Araucaria Bidwillii.—Several of the conifers (*Juniperus Sabina* L.,¹ *Cupressus arizonica* Green,² *Sequoia gigantea* (Lindl.) Dee,³ and *S. sempervirens* (Lamb) Endl.³) have been reported as susceptible to crown gall caused by *Pseudomonas tumefaciens*. *Araucaria Bidwillii* is another conifer that has been artificially inoculated with *Ps. tumefaciens* from peach. *A. Bidwillii* is indigenous to Australia, but is popular in California and Florida as a park and lawn tree.

The artificial aerial galls on *Araucaria Bidwillii* were 5–30 mm. in diameter and nearly spherical. The surface of the galls (Fig. 1, C) was almost smooth, but sometimes was characteristically sculptured by small, regular, angular, brownish areas, probably caused by unequal growth and

TABLE 1.—Results from five-puncture inoculations with *Pseudomonas tumefaciens* on *Araucaria Bidwillii* in 1935

Date of inoculation	Number of galls	Diameter in millimeters
January 3	2	5–25
February 23	2	10–30
April 6	3	5–20
April 16	1	5
May 18	0	0
May 24	0	0
July 5	0	0

¹ Savin (*Juniperus Sabina*). U. S. Dept. Agr. Bur. Plant Indus., Plant Dis. Rptr. Sup. 81: 133. 1931. (Mimeog.)

² Brown, J. G., and M. M. Evans. Crown gall on a conifer. *Phytopath.* 23: 97–101. 1933.

³ Smith, C. O. Crown gall on the Sequoia. *Phytopath.* 25: 439–440. 1935.

tension in the tissue. Inoculations on a rapidly elongating internode gave negative results, the opposite of what was to be expected, and another test should be made. The largest galls were produced on trunk tissue (more than one-year-old) of a small tree growing in the lath house (Fig. 1, A). Galls also were made (inoculation of April 6, 1935) on last season's growth

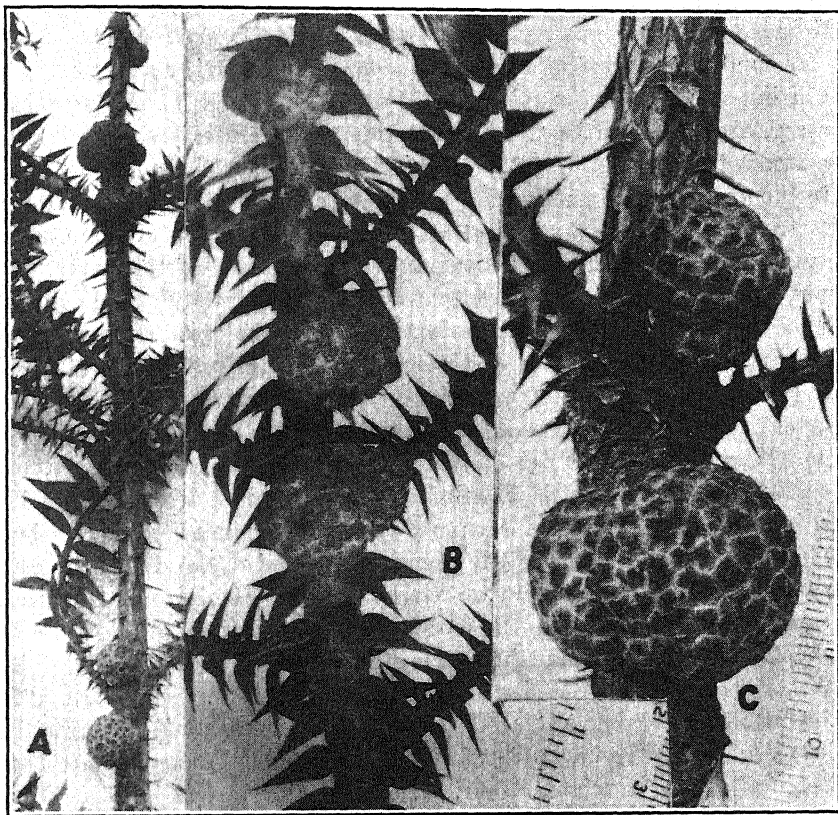


FIG. 1. Artificial inoculation on *Araucaria Bidwillii*. A. Galls (much reduced) of two different inoculations on a small tree growing in the lath house: at top, three galls developing from inoculation of January 3, 1935; at bottom, two galls resulting from the inoculation of April 6, 1935. B. Galls on a small branch of a street tree inoculated February 23, 1935. C. Two galls that were illustrated below in A. These are photographed slightly enlarged and show the characteristic breaking up of the surface.

of a lawn tree (Fig. 1, B). The tabulated results suggest that *A. Bidwillii* is fairly susceptible to crown gall and that the inoculations made in January to April gave positive results, while those made later were negative. The causal organism was reisolated from the galls made on April 6.—CLAYTON O. SMITH, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

BOOK REVIEW

Wollenweber, H. W., and O. A. Reinking. *Die Fusarien—ihre Beschreibung, Schadwirkung und Bekämpfung*. VIII. 355. 95 figs. Paul Parey, Berlin, 1935.

In this book the genus *Fusarium* is treated in the most comprehensive manner, and thus, for the first time, is brought together essentially all data of importance previously published on the subject. This is especially so in regard to description and classification and the diseases caused by or associated with *Fusaria*.

In the preface and introduction are given the high points of the subject, of its treatment in this work, and of the most promising further studies. The principle adopted as to the continuation of the use of the name "*Fusarium*," even after many of the species have been definitely connected with their perfect stages, is of special interest to plant pathologists. The authors consider such use of the name of the imperfect fungi justified because for many of the fungi the perfect stages are still unknown and, therefore, it is important that these fungi should be accurately known in their imperfect stages. However, in every case in which an Ascomycete is definitely known to possess a similar or identical conidial stage, a detailed description of it is given immediately after the *Fusarium* with similar or identical morphology.

The first part of the book (141 pages) is devoted to the description and classification of *Fusaria*. Here the treatment of the subject is essentially the same as that presented in the more recent publications of Wollenweber and Wollnweber in co-authorship with other workers. (See Wollenweber, H. W. *Pyrenomyceten-Studien* I and II. *Angew. Bot.* 6: 300–313, 1924; 8: 168–212, 1926. Same author, *Fusarium-Monographie*. *Z. f. Parasitkde* 3: 269–516, 1931. And same author with others, *Fundamentals for Taxonomic Studies of Fusarium*. *Jour. Agr. Res.* 30: 833–843, 1925). There is only one material deviation from the previously adopted nomenclature in the literature just cited, namely, that some species of *Fusarium*, previously described as "forms," are now given varietal names, when they are known to cause specific plant wilt. In this part of the text a general discussion of the genus *Fusarium*, and other genera closely related to it or identical with it, is followed by a list of all *Fusaria* now recognized, with corresponding Ascomycetes, the latter belonging to the following genera: *Nectria*, *Calonectria*, *Gibberella*, and *Hypomyces*. Then is given a list of the sections, 16 in all, with the Ascomycetes and the *Fusaria* belonging to each section; and this is followed by a classification key for the sections. Finally, each section is treated separately, with classification keys for all *Fusaria* known in each of the sections, and after each of the keys are

described all of the fungi, first under their *Fusarium* name and then under the name of the Ascomycete connected with it, whenever the latter is known. In the systematic portion of the book are described 65 species, 55 varieties, and 22 forms. All the numerous other names previously given are reduced to synonyms.

The larger part of the text, from page 142 to 302, is devoted to the plant diseases caused by or associated with the *Fusaria*, and their control. The diseases of feed and food crops are presented under the generic names of the hosts and the entomophthorous *Fusaria* under Citrus, where these fungi are of greater economic importance. Here, as in the first part, the subject is covered in a thorough manner, apparently without the omission of anything of importance.

Pages 302 to 316 are devoted to the *Fusaria* occurring on other fungi and on living animals, on raw materials and products of plants and animals, and also in soil and water. The next 22 pages present a complete list of the *Fusarium* synonyms and homonyms.

The entire presentation of the subject is excellent, and the material is most conveniently arranged and well illustrated. The book will be extremely helpful to plant pathologists and mycologists interested in any way in this numerous and important group of fungi.—C. D. SHERBAKOFF, University of Tennessee, Knoxville, Tenn.



HERMAN JOHN NINMAN

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HERMAN JOHN NINMAN

1876-1934

S. B. FRACKER

With the death of Herman J. Ninman on October 24, 1934, phytopathology lost one of those consistent and unassuming workers to which every field of science owes so much. Developing from a background in which the forests of northern Wisconsin were an important and ever-present part, he became associated with the white pine blister-rust work almost immediately after its discovery in the Middle West, and for the 17 years from that time until his death he was engaged in various research, survey, quarantine enforcement, and field-control activities connected with the suppression of that disease.

Mr. Ninman was born in Belle Plaine, Wisconsin, on May 4, 1876. His successful efforts to secure even a common school education represented a bitter struggle during which it was necessary for him to be employed continuously in various laborious jobs, which occupied all his time outside the schoolroom. Surmounting these difficulties, he completed a course in the Shawano High School, in 1900; and from then until 1905, he taught in the rural and grade schools of Shawano County. He attended Oshkosh Normal School, Oshkosh, Wis., in 1905, and the State Normal School at Stevens Point from 1906 to 1909. During 1909-10 he was principal of the Melrose (Wis.) High School. This was followed by three years' attendance at the University of Wisconsin, where he was graduated, receiving the degree of Bachelor of Science in Agriculture in 1913. He acted as principal of the Tripoli High School for a year and a half (1914-15) and later returned to the University for postgraduate work, receiving a Master's degree in plant pathology in 1917.

From February 1 to April 30, 1917, Mr. Ninman was employed by the Federal Bureau of Plant Industry as assistant in Tobacco Disease Investigations. That same season the first large-scale suppressive activities directed against the white pine blister rust, which had been found in the north-western part of the State the year before, were begun, and Mr. Ninman became a member of the scouting crew. A brief period as nursery inspector in the office of the State Entomologist of Wisconsin followed. On December 16, 1918, he received a permanent appointment with the Bureau of

Plant Industry of the U. S. Department of Agriculture as field assistant in white pine blister-rust control and quarantine enforcement. His training in plant pathology, his own personal experience in blister-rust control, and his knowledge of the people and of Wisconsin conditions made him particularly successful in that work, and he had the responsibilities of State leader of the project for about 12 years. In 1928 he visited Germany and made a series of careful observations on silvicultural methods, the regional adaptation of tree species, and land utilization. During the last 4 years of his service he was in charge of the research phases of the blister-rust-control project in Wisconsin, except for two brief periods of assignment to quarantine enforcement and to the inspection of white-pine-growing nurseries. Death occurred at Waukegan, Ill., only a few hours after he had become ill while engaged in that locality in field work for the Federal Bureau of Entomology and Plant Quarantine.

Five published papers bear Mr. Ninman's name as author; they are White Pine Blister Rust. Wisconsin Hort. 13: 106-108. 1923.

The Farm Woodlot in Wisconsin. Wisconsin Farmer 56: 353, 361-363. 1927.

Effect of pasturage on white pine reproduction and on timber quality. Jour. Forestry 25: 549-554. 1927.

Notes on European forestry. Jour. Forestry 27: 878-881. 1929.

White pine blister rust control. Wisconsin State Dept. Agr. Bien. Rept. (1927-1928) Bull. 98: 114-117. 1929.

Mr. Ninman's contributions to the development of white pine blister-rust control are much more extensive than this brief bibliography indicates. In the *Blister Rust News*, a mimeographed monthly letter widely distributed among those interested in the protection of white pine, we find that Mr. Ninman contributed 5 articles in 1924, 3 in 1925, 2 in 1926, 4 in 1928, 3 in 1929, 1 in 1931, 1 in 1932, and 3 in 1933. The subject matter ranges from general statements on the Wisconsin blister-rust situation for the season concerned to reports on the rooting of *Ribes* after eradication, the susceptibility of *Ribes missouriense*, the range of the Hudson Bay currant, and the red squirrel as an assistant in blister-rust control, and also includes a number of comments on the possible spread of blister rust by tourists transporting white pines.

In addition, Mr. Ninman is author of some 25 office reports, some of which relate to pine surveys in Wisconsin and Nebraska, and others to the results of the establishment of experimental plots in which various methods of eradication were tested. Among the subjects covered are: Types of tools and their efficiency; the methods of marking the strip lines in crew eradication; the comparative effectiveness of hand pulling and pick pulling; the disposal of uprooted bushes; the average annual growth of *Ribes*

branches; the effect of Ribes removal on game; the survival and growth of Ribes seedlings, and a general report on Ribes ecology.

Mr. Ninman labored throughout his life under physical handicaps and in his early years secured his education by overcoming severe obstacles and without having those surrounding local incentives for endeavor which constitute a considerable part of the inspiration for most of those who are interested in science. His unassuming altruism was reflected not only in his scientific work but also in his personal character, with the result that only a few of his closest friends knew of the extent to which he was continuously helpful in providing moral and financial encouragement to relatives seeking an education and to others with whom he came in contact who were in need.

THE PINEAPPLE ROOT SYSTEM AS AFFECTED BY THE ROOT-KNOT NEMATODE¹

G. H. GODFREY²

(Accepted for publication June 4, 1935)

This paper on the pineapple *Ananas comosus* (L.) Merr. as affected by the common root-knot nematode *Heterodera marioni* (Cornu) Goodey gives a detailed description of the symptoms produced on pineapple root systems, and accumulated information from experiments and observations on the changes produced in the root system by the varying population of nematodes in the soil. Figure 1, A represents a typical condition of severe root knot in the pineapple. The circumstances under which such a condition comes about have an important bearing on the understanding of the relationship of original soil-nematode populations to plant growth and yield, and, consequently, on the significance of the degree of efficiency of any particular control measure.

The question arises as to just how many nematodes are necessary to bring about immediate retardation of growth in an individual root. How near to the root must this population be to produce this immediate effect? How large a total population in the soil around the plant is necessary to bring about an early detectable effect on the plant growth? What is the course of events whereby a plant obtains an early vigorous start and, later, in its growth, shows heavy nematode infestation? These questions are answered, in part at least, in the present paper, by the results of specific investigations and extensive observations.

LITERATURE REVIEW

Complete understanding of the subject matter demands some knowledge of pineapple culture. Three papers are of outstanding value in this connection—those by Henrickson (25), Vosbury and Winston (37), and more recently the very comprehensive book on the pineapple by Johnson (28). A number of general treatises on tropical and subtropical agriculture include sections on pineapple, notably Barrett (1), Bester (2), Bruttini (4), Fenzi (12), Noter (31), Semler (32, *Bd.* 2), and Wohltmann (38). Study

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² Grateful acknowledgement is made to Dr. R. N. Chapman, Director of this Station, for helpful criticism and advice, and to the members of the Nematology staff, particularly Miss Juliette Oliveira and Mr. Charles Sawicki, for routine phases of the research work. The writer takes full responsibility for his interpretation of the experimental results.

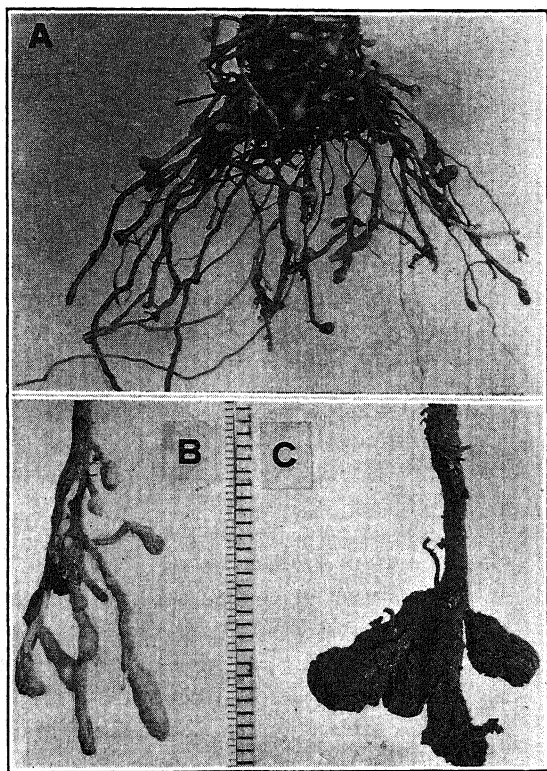


FIG. 1. A. Extreme case of root knot in pineapple, typical of what is to be seen in fields badly infested with the nematode, *Heterodera marioni*. Most of the galls shown are terminal, with a few roots showing nonterminal swellings. About $\times \frac{1}{3}$. B. and C. Typical cases of brooming of roots of pineapple brought about by heavy infestations and reinfestations by the root-knot nematode. B. A young branch root heavily infested. C. Original root end and 3 branches, all heavily infested and beginning to break down from invasion by secondary organisms. Magnification indicated by millimeter scales.

of any of these articles gives a general idea of special methods of propagation, culture, etc. Bowers (3) has written at length on the root system of the pineapple plant and has included general statements on the effects of nematodes and other root parasites. Godfrey and Oliveira (20) illustrate with photographs the relationship of the root-knot nematode in different stages of development to individual roots. Collins and Hagan (10, 22, 24) give accounts of studies of effects of nematode inoculations on several pineapple varieties, with detailed statistical analyses of varietal differences in amount of visible infection and in reaction to infection. Godfrey (17) gives a statistical analysis of the effects of different initial nematode populations, as brought about by chemical treatments, on pineapple plant growth and yield.

THE NORMAL ROOT SYSTEM OF THE PINEAPPLE PLANT

As has been suggested by L. R. Jones, of the University of Wisconsin (intermittently consultant at this Station), the various types of abnormality of the pineapple root system cannot be fully understood until the normal root system is thoroughly understood. Much still remains to be determined in this regard, particularly, concerning its physiological aspects. A general description is included here to facilitate the reader's comprehension of the abnormalities brought about by the root-knot nematode. The normal root system comprises many roots (approximately 100) ranging from 1 to 2½ mm. in diameter. They vary in length from 1 to 2 in. to as much as 3 ft. When young, the roots are white and covered in the usual region back of the root tip with root hairs. They later become tan, and still later brown and woody, losing their root hairs. The first roots to appear after planting arise from root primordia that develop while the planting materials are still attached to the mother plant. When such materials (slips, crowns, or suckers) are first set in the ground, conditions being favorable for growth, this initial root system develops from a region ½ to 1½ in. long on the periphery of the base of the stump or central stalk of the planting material. The initial vigor of plant growth depends largely on that of this first root system. Any condition detrimental to the development of a good early root system results in correspondingly poor plant growth.

Subsequent growth of the plant brings about the initiation of new root primordia in the axils of the new leaves as they attain full development. These grow into the soil and become the root system of the plant. Sometimes new roots appear gradually, one or more at a time; again, a large number may develop and grow into the soil at one time, followed by a period without new root development. The conditions governing new root development are not fully understood. Large branches develop occasionally on main roots. Branch rootlets arise abundantly throughout the length of the main roots, most of them only about ½ mm. wide, from 5 to 20 cm. long, very irregular, and much branched. The history of the development of the pineapple root system is of prime importance to the study of the root-knot problem.

The pineapple belongs to the family of "air plants," the Bromeliaceae. In its growth it frequently manifests its likeness to the other members of the family. It is remarkably tenacious under unfavorable conditions, particularly shortage of soil moisture. Plants with very meagre root systems frequently not only continue to live but to grow and bear. The new axillary roots grow round and round the plant stump, beneath the leaf bases, and undoubtedly function as do the aerial roots of other Bromeliaceae, *e.g.*, *Tillandsia*, by absorbing moisture from light rains and dews. Such roots fre-

quently serve to carry a plant with a very poor soil-root system through to fruiting, particularly if fertilizers are applied by "base-leaf feeding," a method commonly used in pineapple culture.

The pineapple plant must be recognized, then, as having a root system very different from those of the usual agricultural crops. In attempting studies on factors that bring about abnormality in the root development, this fact must be taken into consideration. The very obvious symptoms of abnormal growth induced by the root-knot nematode have led to special attention to its effects on the plants.

The size of the gall is limited definitely by the normal limitation in root diameter. Once a gall is fully developed as the result of a primary invasion by nematode larvae from the soil, it no longer increases in diameter. This type of reaction to nematodes is inherent in the monocotyledonous plants. It occurs with sugar cane *Saccharum officinarum* L.: (9, fig. 28), with banana *Musa sapientum* L., with sorghum *Holcus sorghum* L., with *Syntherisma chinensis* Hitch., and with other grasses sometimes seen heavily infested with nematodes. With dicotyledonous plants, continued development in size of a gall may occur following the development of successive

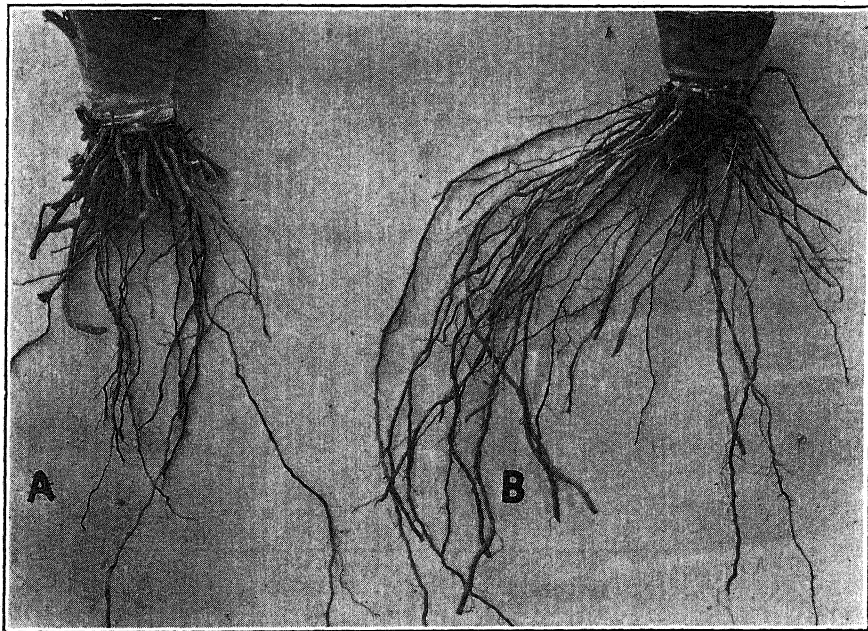


FIG. 2. A. Delayed heavy *Heterodera* infestation of the pineapple root system. Note that the first roots that developed were relatively free and that the plant became well established in the ground, but that the new roots are nearly all heavily infested. B. Plant of the same age, entirely free from infestation, shown for comparison. Greatly reduced.

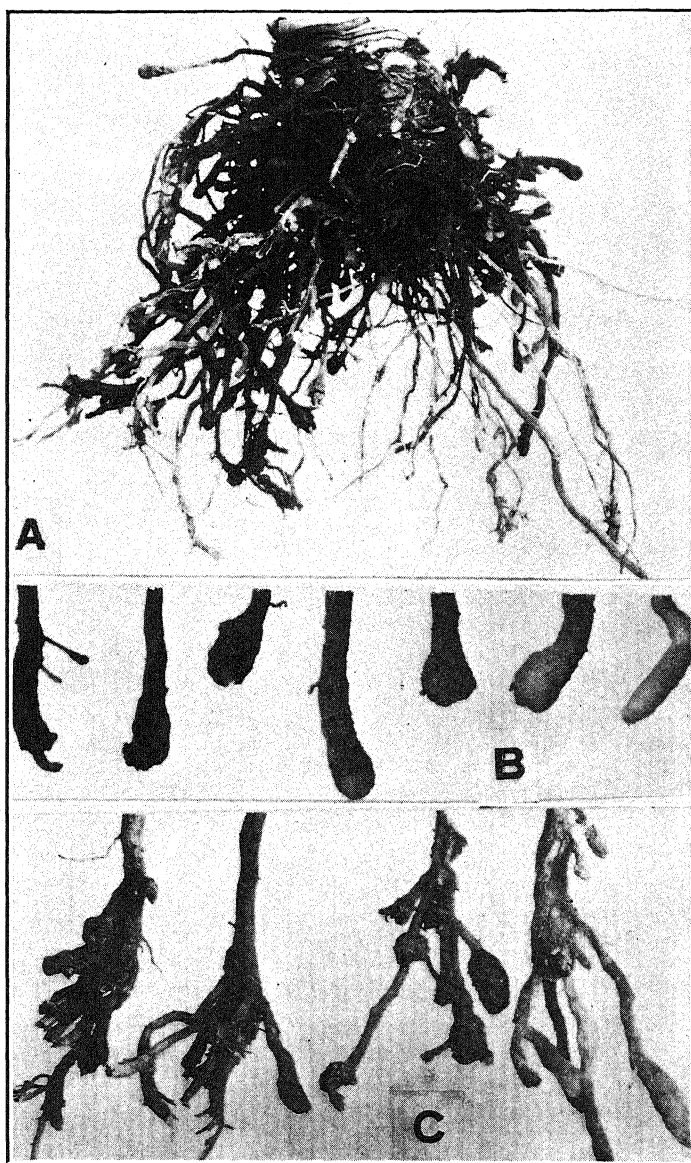


FIG. 3. A. Pineapple root system heavily infested by the two nematodes, *Heterodera marioni* and *Anguillulina pratensis*. The darkened roots and root tips are invaded by the *Anguillulina* in great abundance. B. and C. Typical pineapple root terminal galls: B, simple, and C, much branched, both types showing progressive stages of breakdown from right to left, due primarily to heavy invasion by *Anguillulina pratensis*. The normal reproduction of *Heterodera marioni* does not occur in such cases. A, greatly reduced; B and C, about natural size.

generations of nematodes, by virtue of the continued meristematic activity in the root.

SYMPTOMS OF ROOT KNOT IN THE PINEAPPLE PLANT

General symptoms of nematode root knot are well known, since most of the pertinent literature contains illustrations depicting them. In pineapple the symptoms are somewhat distinctive. Definite galls occur, but they are never so large and complex as those found in tomatoes and other vegetables and many field crops (13, *illus.*). The most evident signs of the disease are the club-shape terminal galls on the main roots, usually 3 or 4 times the diameter of the healthy root, and often somewhat flat on the end. Newly developed terminal galls are white at the tip, grading to yellowish and then the normal brown of the adult root. Old galls become brown throughout their length and often present a coarse network of lateral, or end cracks. Figures 1, 2, and 3 represent typical terminal galls. Frequently, in heavy soil infestation, 90 to 100 per cent of the roots show this symptom. Figure 1, A, illustrates a case of extreme infestation of the entire root system.

Main root terminal and non-terminal galls accompanied by frequent "brooming," and galls on branch rootlets, then, constitute the visible signs of the disease in the pineapple root system. The effects of such infection on the aerial portion of the plant are those commonly associated with root knot in general, *i.e.*, a dwarfing of plant growth, accompanied by a somewhat unhealthy color and some dying back of the leaves, and reduction in yield. These effects occur in proportion to the severity of infestation. Such symptoms, however, may result from a variety of causes. Therefore, above-ground condition of the plant is not, alone, a reliable criterion on which to base the presence or absence of nematode root knot. It must be added, however, that the experienced eye often can thus detect with a reasonable degree of certainty the heavily infested spots in a field. The sequence of developmental changes in a pineapple root system has been demonstrated by a series of experiments, here presented in detail, inasmuch as they, together with other specific observations, are basic to an explanation of what occurs during the life of the pineapple plant.

Besides the terminal galls, nonterminal fusiform enlargements occur on the roots, varying from slight irregular swellings in the woody roots to large distinct galls, somewhat smaller than the usual terminal galls. The smaller ones can not always be identified as such without dissecting the roots and finding the nematodes or the distinctive signs of nematodes having been present. Indeed, roots frequently are found that show no external evidence whatever, and that contain one or more mature females with egg masses, firmly imbedded in the tissues.

Another symptom associated with heavy infestation of the plant is an abnormally profuse branching of the roots approaching that of a broom formation. Figure 1, B and C, and figure 3, C, are typical cases of this, a condition resulting from cessation of growth of a root terminal with consequent stimulation of development in the region of branching back of the root tip. When the plant is still vigorous such branches often become as large as the original root terminal. Where soil infestation is heavy they soon become infected and cease growing, except as they produce several orders of new branches. The typical gall, however, must be sought as the distinctive sign of the nematode, for such "broom" effect may be caused by certain arthropods, by fertilizer injury of the root terminal, or other causes.

Still another symptom is the development of much smaller galls on branch rootlets. These are similar in appearance to those seen on small

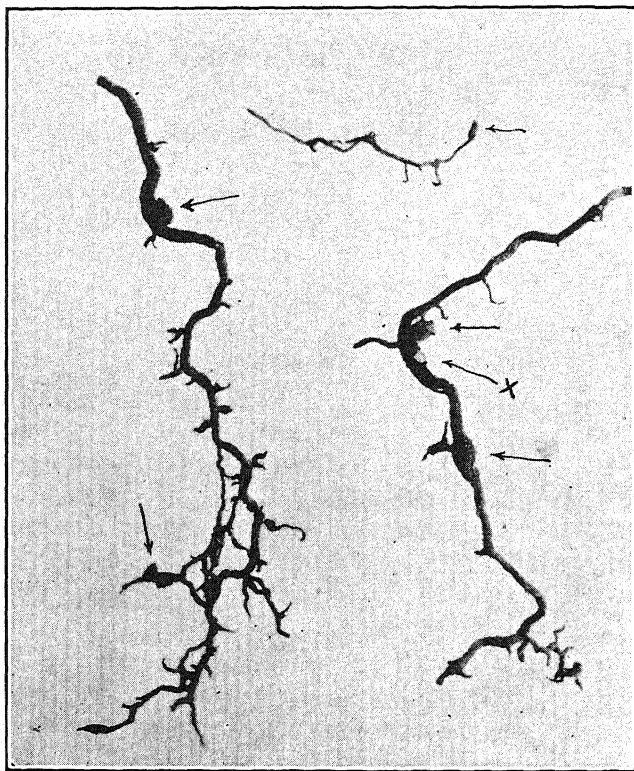


FIG. 4. Small scattered primary infections in branch rootlets of a pineapple plant not yet showing any galls in the main roots. The protuberances, indicated by arrows, on the sides of the roots are *Heterodera* egg masses. X, surface of a spherical female nematode, the egg mass having become dislodged. Magnified about 4 diameters.

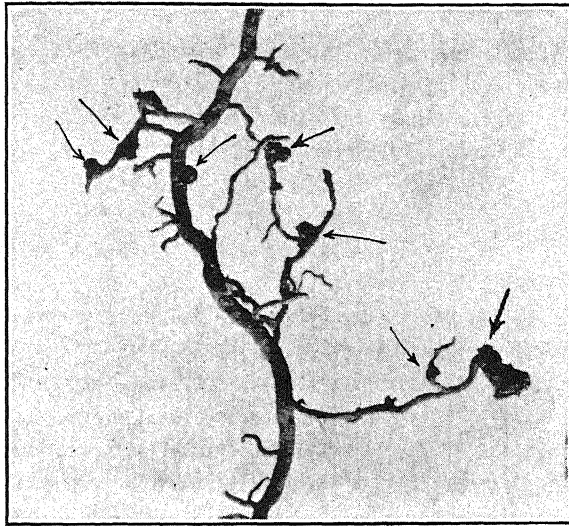


FIG. 5. A small portion of a branch rootlet of pineapple, with small galls and egg masses, (indicated by arrows), produced probably by the progeny of one or more generations, from a light original soil infestation. These galls are very small. If those shown in figures 1, B and 3, B and C were magnified to the same extent they would appear about 2 inches long. The new generation of nematodes evident here is sufficient in numbers to produce a terminal gall on a new main root. Magnified about 4 diameters.

roots of many of the common host plants of the nematode, such as *Begonia* sp., tobacco, and tomato, but are often overlooked in a superficial examination to determine presence or absence of infestation. They are about equally abundant as terminal and intercalary galls and are proportional to the size of the roots on which they occur, frequently being very small. Figures 4 and 5 are typical cases of such galls. In comparing these illustrations with those of main-root galls the difference in magnification should be taken into consideration.

INOCULATION EXPERIMENTS

Experiment 1

Relation of Position of Primary Inoculum to Primary Infection in an Individual Root and to Subsequent Progress of the Disease.—In January, 1931, small root-observation boxes (11) of sterilized soil were planted to slips of several different pineapple varieties. These become well established, as was manifest from root development visible through the glass sides of the boxes. Triplicate inoculations were then made at points 1, 2, and 3 inches to one side of a root terminal and $\frac{1}{2}$ inch below it, in the form of 5 recently collected egg masses at each point. The roots selected for inocula-

tion were as far as possible from the center in order to reduce the chance for closer proximity of the inoculum to another root buried beneath the surface. For comparative purposes, a second series of inoculations was made, usually replicated 10 times, in which the inoculum was placed directly at the tip of the root. At the time of inoculation, sketches were made to scale of the visible root systems of each box, showing the exact positions of the root terminals. At intervals thereafter observations were made and drawings inserted of the subsequent development of the inoculated roots and of new-grown roots. About 9 months after inoculation a final observation was made on plants removed from the boxes. At this time a complete gall count was made in order to record the extent of progress of the disease. Such final gall development was clearly the result of successive infections from the gradually spreading progeny from the introduced nematode eggs.

As the experiment progressed, it became evident that all conditions were not controlled with the desired adequacy. Slow availability of larvae, because of slow and irregular hatching of eggs, often made it possible for a particular root to grow away from the point of inoculation and thus escape early heavy infection. Complete absence of infective material from the rest of the box made this escape more probable. More definite results probably would have been obtained had enough newly hatched larvae instead of unhatched eggs been applied as inoculum. It is possible that watering may have moved the inoculum away from the point of application, though this movement could have occurred only after hatching. There was evidence that in 2 cases out of the 24 of 2- and 3-inch inoculations, another root was buried in the soil nearer than the marked root, thus vitiating otherwise fairly uniform results. For some unexplained reason, 21 of the 78 boxes inoculated were negative throughout for infection. Four out of 42 of these were in the direct-contact inoculation class, 5 of 12 in the 1-inch class, 4 of 12 in the 2-inch, and 8 of 12 in the 3-inch class. All of these were eliminated from the final analysis of results, though the percentages of positive infections were all in favor of the close inoculations.

Regardless of the unsatisfactory features of the test, which can be considered as alike for all lots, certain outstanding differences developed in percentages of inoculated roots showing early initial infection and in total infections produced at the end of the 9-month period. No striking differences between pineapple varieties were evident. For this reason and for the sake of simplifying the report, all are lumped together for the analysis of results (Table 1). Had the 2 exceptional cases, where the presence of a near-by buried root was indicated, been eliminated, the final main-root gall count for the 2- and 3-inch classes would have been 7 and 6, respectively, instead of 13 and 16.

TABLE 1.—*Relation to infection of pineapple roots by Heterodera marioni of position of inoculum with reference to root tips*

Distance of inoculum, inches	Number of plants	Early initial infections			Final infections, galls produced, (av. per box)	
		+	-	% +	Main roots	Branch rootlets
0	37	27	10	73	50	43
1	7	4	3	57	34	57
2	8	0	8	0	13	22
3	4	0	4	0	16	9

Plant-by-plant examinations led to other significant observations. In all cases there was early appearance of small galls on lateral rootlets, followed considerably later by infection of new roots. Roots inoculated directly at the tips showed a high percentage of early infection, whereas roots inoculated at points 2 or more inches away showed none whatever. In general, as shown by the table, plants in which early infection was visible had a significantly higher ultimate main-root gall count. The infections on branch rootlets clearly played an important part in building up the nematode populations. Superficial egg masses appeared abundantly on such rootlets, and the larvae released therefrom were largely responsible for the later infections in main roots. The final rootlet-gall count does not present the entire picture in this respect, for the rootlet system was constantly changing, and many of the early galls disappeared entirely before the final count was made.

Conclusions from this experiment are briefly as follows:

(1) A high percentage of early heavy infection of observed roots did not occur except where those roots grew within 1 inch of the point of inoculation. In other words there was not any striking chemotropic response of nematodes over a distance as great as 2 inches. Their movement appeared to be random, rather than directional.

(2) Early heavy infection of one or more roots was necessary to bring about relatively heavy infection of the entire root system within 9 months.

(3) Failure of heavy primary infection to occur permitted a scattering of the introduced nematode population, so that insufficient numbers were present at any one place for the production of early marked symptoms.

(4) Where the primary inoculation became scattered, any later infections resulted from scattered small infections on branch rootlets. These gradually built up a new population sufficient to infect large roots.

Experiment 2. *The Relation of Magnitude of Initial Inoculum to Development of Terminal Galls in Pineapple Roots.* In July, 1932, several root observation boxes of sterilized soil were planted to slips of Smooth Cayenne

pineapple. When the plants were well established, inoculations were made directly at root tips with counted numbers of newly hatched *Heterodera marioni* larvae, procured by the method described in another paper (16). Immediately after inoculation the exact positions of the ends of both inoculated and noninoculated roots were marked on the glass fronts. Thereafter daily observations were made on the extent of root growth, in millimeters. Progress of root-knot symptoms on individual roots also was noted. It was the objective of this experiment to determine the minimum number of larvae that would result in the stoppage of forward growth and the development of a terminal gall.

To make such a determination, it was necessary to fix upon a definition of a terminal gall that would meet the peculiar conditions of this particular experiment. Under actual field conditions, where there is abundant soil infestation, a root may encounter a constant supply of larvae, and thus be subject to continuous new invasions. Exactly this condition has been illustrated by Godfrey and Oliveira (20). Their figure 11, A, shows what is in all probability a true terminal gall, with newly invading larvae down to the very tip. In the present experiment the inoculum was placed in one spot only, directly on the root tip. If, by vigorous growth, the growing point were able to escape too copious invasion for a day or two, it would reach non-infested soil and then normally continue to grow, in spite of a temporary retardation, thus producing not a terminal, but a nonterminal gall. In taking the daily growth records in this experiment it was found that just this was taking place. While the rate of growth was greatly reduced by abundant infection, growth did continue; and, failing a continuous population of nematodes in advance of the root, it usually was resumed at a greater rate after a few days, or a branch root developed that continued growth without interruption. The average rate of growth of a normal, noninfected root was about 8 mm. per day. It was arbitrarily decided that where the rate of an infected root was reduced to 2 mm. or less per day the resultant gall would be classed as terminal on the assumption that, had a continuous supply of larvae been present in the same degree of infestation the growth probably would have been stopped.

As with Experiment 1, certain irregularities occurred in this experiment, which required due allowance in preparing the tabulation of results. It was noted that, regardless of magnitude of inoculation, the first day's growth, in comparison with that of subsequent days, was always high. This verified the earlier observations of Godfrey and Oliveira (20) that the effect of infection is not manifest until after the first day. It was, therefore, necessary to eliminate the first day in calculating daily averages. As a rule, the following 7 days' growth was made the basis of calculations. Some roots were early observed to die from other causes. Some few, obviously clear excep-

tions, grew normally, or nearly so, without marked sign of infection, due perhaps to escape of the bulk of the larvae from the original site to other roots in the vicinity or to being washed away. Some roots, after inoculation, grew into the interior of the box, so that further observations were impossible. All such distinct exceptions are eliminated in the calculations. In none of the magnitude-of-inoculation classes was the number of cases sufficiently high to eliminate rather wide variations. Consequently, probable errors of the averages are high and differences are not so significant as might be expected with larger numbers.

The results of the experiment are presented in condensed form in table 2, where terminal galls as defined above are recorded. Nonterminal galls are those in which a distinct gall is evident, but growth was retarded only temporarily.

TABLE 2.—*Results of inoculations directly at pineapple root tips with counted numbers of Heterodera marioni larvae*

Series	Size of inoculum	Number of cases	Number discarded	Rate of growth per day mm.	Percentage with terminal galls	Percentage with nonterminal galls
I.	0	12	0	8.12 ± 0.25	0.0	0.0
	50	7	3	3.55 ± 0.48	28.6	14.3
	100	6	4	2.14 ± 0.54	50.0	50.0
	150	8	1	1.78 ± 0.26	62.5	25.0
	250	7	2	1.34 ± 0.27	85.7	14.3
	500	8	1	1.43 ± 0.36	85.7	14.3
	1000	3	0	1.12	100.0	
	2000	3	0	0.95	100.0	
	3000	3	0	0.75	100.0	
	4000	3	0	0.90	100.0	
	5000	5	0	1.38	100.0	
II.	0	5	0	8.0 ± 0.6	0.0	0.0
	100	6	0	2.88 ± 2.18	50.0	16.7
	150	6	1	2.24 ± 0.39	66.7	16.7
	200	4	1 ^a	1.47 ± 0.20	100.0	0.0
	250	7	0	1.79 ± 0.14	50.0	50.0
	300	6	1 ^a	1.30 ± 0.14	100.0	0.0

^a Discarded because of almost complete escape from infection, as indicated by growth almost equal to that in the controls (6.7 mm. and 5.4 mm., respectively) due to some uncontrolled factor. All other cases listed were discarded because of their early death or of growth of tip into interior of box.

Examination of this table discloses that heavy retardation of root growth in 50 per cent or more of the cases, with virtual formation of terminal galls, occurred only in those classes in which the inoculum consisted of 100 or more

larvae. The differences in root growth in the classes of 100 and over are not statistically significant on the basis of present data, though the fair consistency of the gradual increase in percentage of roots showing terminal galls up to inoculations with 1000 larvae would make it appear that larger numbers of cases in the classes might make them so. The difference in growth between noninoculated controls and all inoculated classes is perfectly evident. That between the 50 class and the 300 class is $2.25 \pm .50$, the difference being $4\frac{1}{2}$ times its probable error. With the inoculations of higher magnitudes, 1000 and up, a superfluous population for the individual root was present, and sufficient numbers migrated to other roots, where early development of terminal galls occurred.

In this study with known populations of the root-knot nematode and their effects on pineapple roots, a number of difficulties have arisen, inherent in the small size of the organism and the lack of complete control possible with the equipment used, and these have made for lack of exactness in results. Among such difficulties are: (1) The difficulty of getting sufficiently large numbers of cases, for each population used, of active living larvae of the same age and vitality; (2) the difficulty of maintaining complete uniformity in soil moisture content about the root tips; (3) uncertainty as to uniformity in vigor of root growth; (4) lack of complete control in the matter of proximity of other roots; (5) difficulty in confining the larvae within uniformly controlled space limits at the point of inoculation. With continued effort on this problem some of these difficulties can be overcome and more exact results should be obtainable. It is impossible to obtain the degree of exactness of results reported by Chapman (7, 8) with *Tribolium confusum* Duval, for example, in which practically every individual could be accounted for.

Pertinent Data from Other Experiments. In the spring of 1932, 120 5-gallon (20 liter) wooden tubs of steam-sterilized soil, divided into 6 groups of 20 tubs each, were planted to pineapple slips. When the plants were well established, with roots from 1 to 2 inches long, they were inoculated with *Heterodera* larvae in quantities of 4, 20, 100, and 1000 to the plant, in 4 groups, the 5th group receiving 20 egg masses, or, potentially, about 8000 larvae, and the 6th serving as a control. The inocula were placed uniformly at one level, immediately proximate to the growing roots. At the age of 6 months there was no evident difference in plant growth between groups. In the light of the results from experiments 1 and 2, this is not surprising, since in two important respects the conditions did not meet with the requirements for immediate heavy infestation of the root system. In no case was the infective material uniformly distributed through the soil, thus many roots escaped early infection; and, even with most abundant inoculum, the magnitude of soil infestation averaged less than 10 larvae per cu. in., or

less than 1/10 the minimum number necessary for early and complete root infection.

In another experiment, reported by Collins and Hagan (10, 24), with Smooth Cayenne pineapples planted in tubs of 0.65 cu. ft. capacity, and inoculations of 500 egg masses (potentially 200,000 larvae), reduction in root length of 15.2 per cent and in plant weight of 29 per cent occurred, as compared with the controls. In this case, due probably to the fact that the nematodes were not uniformly distributed through the soil, many roots escaped complete stoppage in growth, but the final effect (at 8 months) on root and plant growth was nevertheless a substantial one.

Magistad and Oliveira (29) likewise show a very striking reduction in total root and plant growth brought about by a distinctly high population of nematodes.

These 3 supplementary experiments are reported as practical substantiation of the results to be expected in the light of the findings from experiments 1 and 2.

In another experiment, it was determined that the rate of spread of nematode infestation is very low in the root system of the pineapple plant. With well-established plants growing in sterilized soil, the end plant only was inoculated, in a row of plants spaced 12 inches apart. Seven months passed (with monthly observations) before infection was evident in the adjacent plant. Spread to only the third plant in the row had occurred by the 12th month. The spread in a row of cowpeas, with replantings when necessary, was 8 feet during the 12 months' period. The spread in both cases was by nematode motility and reproduction alone, mechanical agencies being eliminated.

CONSTANTLY CHANGING ROOT SYSTEM OF A PINEAPPLE PLANT AFFECTED BY NEMATODES

Progressive Changes Due to the Nematodes. Under the heading "Symptoms" in this paper a detailed description has been given of root knot in the pineapple plant. For any one plant the details of the picture are constantly changing. A root terminal with a newly developed gall may either continue to grow, resulting in a nonterminal gall, or it may enlarge to maximum size and remain permanently terminal. All nonterminal galls were originally terminal, as stated by Godfrey and Oliveira (20). The continuation or discontinuance of growth is determined by 3 factors, *viz.*, (a), numerical magnitude of the infection, *i.e.*, the number of larvae that have actually entered the growing root tip; (b), vigor of the plant and of root growth at time of infection; and (c) presence or absence of additional larvae in the soil below the root tip. Possibly also, as has been observed, the individual reaction of a root in the way of surface cracking, whether terminal or lateral,

is a factor. If lateral, continuation of root growth is more apt to occur. Some of the present terminal galls may, therefore, become nonterminal. A high proportion of nonterminal galls in any particular plant is usually an indication of vigorous root growth during the period of exposure, or of relatively light soil infestation.

The fact of continued root growth in the case of a nonterminal gall is proof that the root is functioning in the matter of continued movement of water upward from the soil and translocation of elaborated food downward to the root tip. The economy of the plant, therefore, is not greatly hampered. True terminal galls, then, are obviously the more injurious of the two, since they prevent normal root growth and thereby limit its feeding range. The very light infections that occur in main roots and that are scarcely detectable because of the hardening of the root tissues, without obvious gall formation, are virtually of no direct importance to the plant's economy.

Other changes constantly occur. Old roots die, and new ones grow from the axillary root primordia in the plant, and, where infestation is abundant they go through the same stages of gall development. Branch roots arise just above a terminal gall, and likewise may become infected. The branch rootlet system constantly varies. Once nematodes are numerously established in a root system, an actual *surplus* of larvae may be present in the soil at all times. In one experiment, heretofore reported (18), 11 successive plantings of a trap crop beside a heavily infested row of pineapples, each of which removed tremendous numbers of nematodes from the soil, produced no noticeable reduction in the amount of infestation in the pineapple plants, as compared with others in which trapping was not done. With such infestation, the root system presents much the same picture month after month, even though individual roots are constantly changing, unless some external agency has appeared that is capable of making a profound change in the nematode population.

Changes Brought About by Other Organisms. It is not to be expected that, under field conditions, a pure nematode population will be present to act without the influence of other organisms. Sideris (33) and Sideris and Paxton (34) have considered various fungi that kill pineapple root tips. In root study boxes, the writer has observed root tips initially penetrated by nematodes and showing early stages of gall development, later attacked by fungi and killed, completely preventing normal production of a new generation of nematodes. Entire fields have been observed in which terminal root galls become decayed, apparently soon after they are formed. Thousands of plants show the typical appearance of nematode infection (Fig. 1, A) with an abundance of short roots terminated by galls. Most of the galls, however, are brown and collapsed, the older ones being completely rotted internally, and the younger ones soft and watery. Histological examination of such

roots discloses the presence of a fungus permeating the tissues. Nematodes were doubtless the primary invaders, since the root-knot nematode is an obligate parasite and attacks only healthy roots. A tremendous change in normal nematode relations has been brought about by this secondary invader. If conditions, favorable for root growth and unfavorable to the fungus, soon recur, renewed plant growth may take place, with a much reduced nematode menace.

Again, the writer has observed in root-study boxes root terminals initially inoculated with nematodes and showing early stages of gall development, attacked before maturity of the contained nematodes by the larvae of a mycetophylid fly, *Sciara* sp., mentioned by Illingworth (27) as occasionally feeding upon healthy root tips. The result was a hole eaten into the side of the root tip, the hollowing of the tip, and the complete destruction of the nematodes. Healthy branch roots then developed and grew past the old terminal, without attack by nematodes. The garden Symphylid, *Scutigerella immaculata* Say likewise has been observed feeding upon galled root terminals and thereby preventing the propagation of a new generation of nematodes. The root-lesion nematode *Anguillulina pratensis* (de Man) Goffart (*Tylenchus brachyurus* Godfrey, 15) very definitely hastens the breakdown of a terminal gall, as shown in figure 3.

Many natural enemies of the nematode occur in pineapple-field soils. The writer has observed in root-study boxes of field soils a small brownish elongate active mite (species undetermined) actually feeding upon living larvae of *Heterodera marioni*. Dr. Hagan has observed this mite in great numbers in an experimental plot where the nematode was not reproducing so rapidly as was to have been expected in a continuous stand of susceptible cowpeas. Muir (30) has reported a fungus, later referred to (6) as *Microcera* sp., on *Heterodera* egg masses. The writer has found it in great abundance in garden and field soils, and has observed a large proportion of both eggs and newly hatched larvae dead in affected egg masses. Unfortunately, he did not have time to follow up his observations with extensive cultural studies. He also has found an undetermined protozoon parasitizing larvae. Species of the nematode genus *Mononchus*, known to be predators upon *Heterodera marioni*, are abundant in Hawaii, and particularly so in moist localities where the *Heterodera* was remarkably scarce. In laboratory studies, the writer has seen an entire group of 20 *Heterodera* larvae eaten or killed in a single night by one individual *Mononchus papillatus*. Cassidy (5) has described and pictured several species of the genus occurring in sugar-cane fields in Hawaii. Very possibly other natural enemies, not yet observed, occur in pineapple-field soils.

Complete studies of these organism complexes have not been made. It is certain, however, that they frequently influence very greatly the normal course of development of root knot in the pineapple root system.

The Influence of Environmental Conditions.—Environmental conditions play an important part in the survival of the nematode in the soil, and thereby influence the normal course of development of root knot in the pineapple field. Hoshino and Godfrey (26) have shown that the minimum lethal temperature for long exposures is about 41° C. Hagan (23) has determined that such lethal temperatures, sometimes much higher than 41° C., may occur under field conditions to a depth of at least 2 inches and sometimes even to 3 inches, thereby eliminating live nematodes from the upper layers of soil. Godfrey, Oliveira, and Gittel (21) have shown that soil moisture plays an important part in the longevity of the nematode. Under conditions of high relative humidity, which prevents drying, and low free water, which prevents activity, the nematodes may survive in the soil for many months. Under field conditions, this period of survival appears to be even longer than under the artificial conditions set up in their experiments, where survival for over 40 weeks was demonstrated. In long continued clean-fallow, field experiments indicator crop readings on the nematode population of the soil were taken at intervals. While very great reduction of the population was demonstrated, occasional plants continued to show live nematodes in the soil, even after 2 years, and a subsequent planting of pineapples, fairly free at first, later built up a distinct, though not very high, population. Some reinfestation from susceptible weeds was evident after the expiration of the first year. Some was apparently due as well to a heavy wash that occurred from an adjoining infested field. But it is believed that there was some survival of the original population in the deeper soil.

Temperature again plays an important part both in infection and in the length of life cycle of the root knot nematode. Godfrey (14) has given 13° C. as the approximate minimum at which any extensive infection will take place. Tyler (36) has shown that the length of life cycle is increased with the lowering of the temperature below the optimum for development, and decreased by raising the temperature within limits. Consequently, under field conditions, considerable variation in rate of development of the disease occurs, with fluctuations of temperature.

The simultaneous occurrence of other root-infesting organisms, the natural enemies of the nematode in the soil, and unfavorable environmental conditions, then, constitute certain "environmental resistances" that prevent the reproduction of the nematode to its full potential capacity. A major environmental resistance, however, has to do with the host plant itself. In the absence of a host plant the population constantly diminishes. Multiplication occurs only by infection and reproduction in the roots of a susceptible plant, whether it be weed or cultivated crop. Calculating each new egg mass as 400 potential larvae on the average (19, p. 43), only 0.5 per cent of potentially possible reproduction in a spot in the soil would serve to maintain the

population. For example, if any given volume of soil contained 2000 living eggs or larvae at a given time, only 10 of them reproducing to full capacity (granting half of them to be males) would bring the population back to 2000. Commonly the proportion of males is much less than $\frac{1}{2}$; indeed, Tyler (35) has shown that the presence of males is not essential to reproduction, so the potential rate of increase is even greater than that suggested. Again, it has been estimated that under optimum environmental conditions, with absolutely no environmental resistance present at any time (such as nonavailability of susceptible host tissues) the population in one year, starting with one nematode to the square foot, would be sufficient to make a solid layer of nematodes many miles thick over the surface of the field! Fortunately, the resistances come into active play with the first generation.

DISCUSSION

The experiments and observations give sufficiently exact information upon which to base the interpretation of field occurrence of root knot. In Hawaiian pineapple plantations 3 main degrees of plant infection, occurring over large areas, may be specified.

(1) *Early heavy infection* of the primary root system and continuous attack of new roots as they develop, resulting in an early dwarfing effect on plant growth and definite reduction in yield.

Experiments 1 and 2 show that a population of at least 100 larvae to the cubic inch, or 172,800 to the cubic foot, uniformly distributed through the soil, may be considered to be near the minimum number necessary to bring about certain early terminal gall formation on all the primary roots of a pineapple plant. This extent of infestation is not at all unusual in the field. A cowpea root, such as that shown in figure 2 of a previous paper (16), was potentially capable of releasing 80,000 larvae from each inch of its length. The entire root system would have released 1,600,000 larvae into less than a cubic foot of space. Owing to limited acreage with certain companies, pineapple plantings have been made shortly after plowing under a previous heavily infested crop, without the normal intercycle period for nematode reduction. Plantings also have been made following a highly susceptible rotation-crop legume, such as *Sesbania macrocarpa* Muhl. or rice bean (*Phaseolus calcaratus* Roxb.). In all such cases, as one might suspect, infestation of this classification occurs.

(2) *Early mild infection*, not plainly evident on the primary root system, with no marked effect on early plant growth, followed by what appears to be a sudden heavy attack on new roots at from 6 to 8 months after planting.

This type of infestation appears in plantings following an inadequate intercycle period or a rotation crop that has permitted the growth of nematode-susceptible weeds. The soil infestation is not of sufficient magnitude to

retard seriously the growth of the primary root system. The small branch rootlets become infested, however, (Figs. 4 and 5); and, through the course of 3 or 4 generations, they build up a sufficient population to produce terminal galls on the new roots. This accounts for the appearance of such a plant as that shown in figure 2, and for the so-called sudden appearance of infestation in a field that has earlier seemed to be relatively free.

(3) *Light, delayed infection*, with almost complete escape of the first set of roots, with vigorous plant growth and apparently undiminished plant-crop yield, followed by the gradual appearance of the usual root-knot symptoms in increasing abundance, and damage manifest in the ratoon crops.

This is the usual condition in pineapple fields that have received the full two-year rest period for nematode reduction, with clean fallow, or immune rotation crops. The nematodes have been greatly reduced, but not completely eliminated. There is very light infestation or none at all in the upper 6 or 8 inches of soil, and some survival in the deeper layers. The roots, then, must grow into this deeper soil before infection occurs. The course of development of the nematode population is much as in the preceding case, but considerably delayed.

SUMMARY

The normal pineapple root system consists of about 100 main roots of uniform size covered with branch rootlets.

The symptoms of root knot are club-shape, terminal galls; fusiform, non-terminal galls; a "brooming" of root terminals caused by the development of a number of branch roots, and abundant small galls on branch rootlets. Large compound galls do not occur, as they do with most other crops.

The individual details of root galls are constantly changing because of decay of old galls and the development of new axillary roots.

Other root-infesting organisms, including root-rotting fungi and various fauna, play an important part in the changing root-knot situation.

The magnitude and location of primary soil infestation determine whether or not serious initial damage occurs. Experiments show that infestation of the magnitude of at least 100 active larvae located directly at the root tip is necessary to stop the growth of a vigorous root and produce a terminal gall.

Any plant in which essentially every primary root has been stopped by a terminal gall may be considered to have been planted in soil with an initial infestation of at least 100 larvae per cubic inch, or 172,800 larvae per cubic foot, fairly uniformly distributed through the soil.

Where primary infestation is distinctly less than this, many of the roots escape serious injury and the plants become well established. In such cases, however, in later stages of plant growth, heavy infection may occur as the result of the gradual building up of a high nematode population from pri-

mary infections in branch rootlets and small roots. This results in the very common condition of long main roots relatively free from infection, and new short axillary roots heavily infected.

Heavy infection of the initial roots from the start results in dwarfed plant growth, greatly reduced yield, and early failure of ratoons. Delayed heavy infection permits of good initial root and plant growth and good plant-crop yield, with subsequent yields dependent upon the magnitude of the nematode population that develops.

External agencies, not yet thoroughly studied, may vitiate the uniformity of this normal course of the relationship of nematodes to the pineapple plant.

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FLAX SEED-TREATMENT TESTS¹

H. H. F L O R ²

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INTRODUCTION

Seed treatment for flax, for the control of certain seed-borne diseases such as seedling-blight or canker, *Colletotrichum lini* Bolley, and wilt, *Fusarium lini* Bolley, has been advocated since 1910 (1). The formaldehyde-spray treatment was the one most widely recommended but its use never became general. The mucilaginous nature of the flax seed coat made it difficult to apply the formaldehyde solution thoroughly enough to disinfect the seed without the latter becoming sticky and hard to handle. Also, experimental data on the effect of seed treatment on stand, yield, and prevalence of diseases of flax was limited. The discovery and use of seed disinfectants in powdered form has renewed the interest of investigators in flax seed-treatment tests. Schilling (6, 7), Medish and Raro (5), and Eglits (3) have secured an increase in quantity and quality of flax straw and, in some cases, an increase in yield of seed by treating with fungicidal dusts. The most beneficial effects were obtained when seed of inferior quality was used or when seedling diseases were prevalent. Burnett and Reddy (2) obtained beneficial results with the Ceresan dust treatment in 1 year's trials at a number of localities in Iowa. On the other hand, Kletschetoff (4) tested 134 seed disinfectants, including those reported most beneficial by other investigators (3, 6, 7), and found that none gave satisfactory results.

The cooperative tests reported in this paper were initiated to determine the effect of treatment of flax seed on stand, yield, and incidence of disease in the different sections of the principal seed-flax producing area of the United States.

The investigators cooperating in these tests and the stations at which seed-treatment tests were conducted are as follows:

C. C. Allison,
Minnesota Agricultural Experiment Station,
St. Paul, Minn.

R. O. Bridgford,
West Central Experiment Station,
Morris, Minn.

R. E. Hodgson,
Southeast Experiment Station,
Waseca, Minn.

H. E. Morris,
Montana Agricultural Experiment Station,
Bozeman, Mont.

¹ Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

² Associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

R. W. Smith,
Dickinson Substation,
Dickinson, N. Dak.

O. A. Thompson,
Edgeley Substation,
Edgeley, N. Dak.

H. H. Flor,
North Dakota Agricultural Ex-
periment Station,
Fargo, N. Dak.

C. H. Plath,
Hettinger Substation,
Hettinger, N. Dak.

Glenn S. Smith,
Langdon Substation,
Langdon, N. Dak.

J. C. Brinsmade, Jr.,
United States Northern Great
Plains Field Station,
Mandan, N. Dak.

K. H. Klages,
South Dakota Agricultural Ex-
periment Station,
Brookings, S. Dak.

The curtailment of budgets and crop failures due to drought restricted the tests at a number of the stations to 1 year's results. Altogether, seed-treatment data were secured from tests involving 57 different dates of sowing.

MATERIALS AND METHODS

The seed used in these tests was from the preceding year's crop grown at Fargo, N. Dak. It was uniformly treated at Fargo, packaged in envelopes for rod-row seeding, and distributed to the individual cooperators shortly before time for sowing. The standard plot of 3 rod rows was used in these tests, and stand and yield data were obtained from the center row of each plot. In 1931, 1933, and 1934 four plots for each treatment were used, and the 1932 tests had but three plots for each treatment. In 1931, two varieties, Bison (C. I. 389),³ wilt-resistant, and Damont (C. I. 3), wilt-susceptible, were sown during the period recommended for flax seeding at each station. In 1932, 1933, and 1934 only the Bison variety was used and three sowings were made at each station at intervals of about 2 weeks. In 1931 and 1932, the following three treatments were used: (1) The formaldehyde-spray treatment, in which the seed was sprayed with a 1 to 320 solution of commercial formaldehyde at the rate of 1 gallon per bushel, covered over night and then dried; (2) the copper carbonate dust treatment; and (3) the Ceresan (ethyl mercuric chloride) dust treatment. The copper carbonate and Ceresan were each applied at the rate of 4 ounces per bushel. Copper carbonate dust, containing 50 to 52 per cent of metallic copper, was used. In 1933, the formaldehyde treatment was dispensed with at the co-operating stations because of injury resulting from delayed sowing. In the 1933 and 1934 tests at Fargo, the formaldehyde-spray treatment was applied the day prior to sowing. The individual cooperators were not specifically

³ C. I. refers to accession number of the Division of Cereal Crops and Diseases.

requested to make accurate stand counts because it would have required considerable labor at a busy season of the year. However, stand data were taken at a number of stations, and these together with data on yield are given in table 1.

EXPERIMENTAL RESULTS

The failure of any treatment to be decidedly beneficial either to stand or yield is clearly indicated in table 1. The most conspicuous effect of the treatments was the injury that resulted from the use of formaldehyde. This was more pronounced on stand than on yield. Stand counts were obtained from 24 tests in which the effect of the formaldehyde, Ceresan, and copper carbonate treatments and the non-treated check were compared. The stand of plots sown with non-treated seed and seed treated with Ceresan and copper carbonate was better than that of plots sown with formaldehyde-treated seed in 21 tests each; the stand of plots sown with formaldehyde-treated seed was better than that of those sown with non-treated seed in 2 tests and equal in 1 test, and was better than that of plots sown with seed treated with Ceresan and copper carbonate in 3 tests each. The stand of plots sown with Ceresan-treated seed was better than that of plots sown with non-treated seed in 18 out of 28 tests and better than the stand of those sown with copper carbonate-treated seed in 16 out of 28 tests. Plots sown with non-treated seed had superior stand counts to those sown with copper carbonate-treated seed in 15 out of 28 tests.

The effects of the treatments on yield were more variable than were those on stand and the injurious effect of the formaldehyde treatment was less pronounced. There were 47 tests in which the average yield of plots sown with formaldehyde-treated seed was compared with that of plots sown with non-treated seed and seed dusted with Ceresan and copper carbonate. In 15 tests the average yield from plots sown with formaldehyde-treated seed was greater than that from those sown with non-treated seed, and in 32 tests it was less. In 15 tests, plots sown with formaldehyde-treated seed yielded more than those sown with seed dusted with Ceresan; in 30 tests, they yielded less; and in 2 tests, the yields were identical. In 12 tests, plots sown with formaldehyde-treated seed yielded more than those sown with copper carbonate-treated seed; in 34 tests they yielded less, and in 1 test, the yields were identical. There were 55 tests in which the yield of plots sown with non-treated seed was compared with that of plots sown with seed treated with Ceresan and copper carbonate. In 28 tests, the plots sown with Ceresan-treated seed yielded more than those sown with non-treated seed; in 23 tests they yielded less; and in 4 tests, the yields were identical. The plots sown with seed treated with copper carbonate yielded more than those sown with non-treated seed in 27 tests, in 22 tests they yielded less, and in 6 tests the

TABLE 1.—Effect of treating flax seed on yield and stand at 11 stations in Minnesota, North Dakota, South Dakota, and Montana from 1931-34

Station	Variety	C. I. no.	Date sown	Stand in plants per rod row				Yield in bushels per acre			
				Non-treated check	Treated with			Non-treated	Treated with		
					Formaldehyde spray	Ceresan dust	Copper carbonate dust		Formaldehyde spray	Ceresan dust	Copper carbonate dust
Bozeman, Mont.	Bison Damont	389	1931	12.7 ± 1.29	10.7 ± 1.08	10.0 ± 1.01	9.9 ± 1.00
		3	1931	11.6 ± 1.34	8.4 ± 0.97	11.3 ± 1.30	12.3 ± 1.42
	Bison	389	1932	a13.6 ± 1.43	a13.2 ± 1.39	a9.8 ± 1.03	a16.4 ± 1.73
	Do	389	May 10	12.6 ± 0.69	10.0 ± 0.55	12.6 ± 0.69	11.1 ± 0.61
Brookings, S. Dak.	Do	389	May 24	9.1 ± 0.47	6.1 ± 0.32	9.8 ± 0.51	10.1 ± 0.53
	Do	389	June 4	8.8 ± 0.49	7.7 ± 0.43	8.6 ± 0.48	10.5 ± 0.59
	Bison	389	May 2	b99 ± 1.8	b73 ± 1.3	b98 ± 1.8	b105 ± 1.9	3.5 ± 0.17	2.6 ± 0.13	4.4 ± 0.22	3.9 ± 0.19
	Do	389	May 17	b123 ± 3.3	b89 ± 2.4	b125 ± 3.4	b115 ± 3.1	10.7 ± 0.80	7.2 ± 0.54	12.2 ± 0.92	12.9 ± 0.97
Dickinson, N. Dak.	Bison	389	May 7	10.4 ± 0.46	7.0 ± 0.31	10.6 ± 0.47	10.1 ± 0.45
	Do	389	May 20	11.8 ± 1.26	7.8 ± 0.84	11.0 ± 1.18	8.4 ± 0.90
	Do	389	June 1	10.3 ± 0.70	10.7 ± 0.73	11.6 ± 0.79	11.9 ± 0.81
	Bison	389	1931	9.4 ± 0.73	9.7 ± 0.76	9.6 ± 0.75	12.0 ± 0.94
Edgeley, N. Dak.	Damont	3	1931	13.9 ± 0.33	13.8 ± 0.33	14.0 ± 0.34	14.5 ± 0.35
		3	May 16	11.5 ± 0.69	12.8 ± 0.77	12.1 ± 0.73	13.2 ± 0.79
	Bison	389	1932	17.9 ± 0.77	16.4 ± 0.71	16.8 ± 0.72	17.4 ± 0.75
	Do	389	May 2	755 ± 39.0	435 ± 22.6	766 ± 39.8	716 ± 37.2	16.9 ± 0.41	15.9 ± 0.38	17.2 ± 0.41	17.1 ± 0.41
Fargo, N. Dak. (farm soil)	Do	389	May 19	741 ± 24.2	482 ± 15.7	720 ± 23.6	764 ± 25.0	10.9 ± 0.72	9.9 ± 0.65	11.5 ± 0.76	11.9 ± 0.79
	Do	389	June 14	572 ± 25.6	321 ± 14.5	602 ± 27.0	577 ± 26.0	13.0 ± 0.31	13.5 ± 0.32	14.9 ± 0.36	14.7 ± 0.35
	Do	389	1933	708 ± 19.8	602 ± 16.8	726 ± 20.3	749 ± 21.0	16.9 ± 0.80	16.8 ± 0.79	17.4 ± 0.82	14.8 ± 0.70
	Do	389	May 2	683 ± 17.1	619 ± 15.5	633 ± 15.7	648 ± 16.2	6.6 ± 0.30	6.7 ± 0.30	6.4 ± 0.29	6.2 ± 0.28
	Do	389	June 2	4.8 ± 0.37	5.2 ± 0.39	4.1 ± 0.31	4.8 ± 0.37
	Do	389	1934	111 ± 7.8	112 ± 7.8	138 ± 9.7	97 ± 6.8	1.6 ± 0.44	1.0 ± 0.27	1.9 ± 0.52	2.2 ± 0.60
	Do	389	April 28	625 ± 11.9	562 ± 10.7	650 ± 12.3	608 ± 11.5	4.2 ± 0.44	5.0 ± 0.53	4.5 ± 0.47	4.5 ± 0.47
	Do	389	May 12	568 ± 10.8	496 ± 9.4	561 ± 10.7	575 ± 10.9				

TABLE 1.—(Continued).

Station	Variety	C. I. no.	Date sown	Stand in plants per rod row				Yield in bushels per acre			
				Non-treated check	Treated with			Non-treated	Treated with		
					Formaldehyde spray	Ceresan dust	Copper carbonate dust		Formaldehyde spray	Ceresan dust	Copper carbonate dust
Fargo, N. Dak. (wilt-sick soil)	Bison	389	1931 May 12	9.3 ± 0.49	9.7 ± 0.52	9.0 ± 0.48	8.6 ± 0.46
	Damonte	3	May 12
	Bison	389	1932 May 13	284 ± 16.7	206 ± 12.2	329 ± 19.4	279 ± 16.4	6.7 ± 0.48	6.2 ± 0.45	8.0 ± 0.58	8.6 ± 0.62
	Do	389	1933 May 12	544 ± 27.2	470 ± 23.5	469 ± 23.4	496 ± 24.8	11.7 ± 0.84	11.1 ± 0.80	11.1 ± 0.80	10.8 ± 0.78
	Do	389	1934 April 28	285 ± 19.1	248 ± 16.6	328 ± 22.0	273 ± 18.3	11.7 ± 0.75	11.8 ± 0.75	12.2 ± 0.78	11.9 ± 0.76
	Do	389	May 12	492 ± 27.6	450 ± 25.2	408 ± 22.8	436 ± 24.4	5.7 ± 0.39	6.1 ± 0.42	5.5 ± 0.38	5.7 ± 0.39
Hottinger, N. Dak.	Do	389	May 26	496 ± 25.8	385 ± 21.2	508 ± 28.0	519 ± 28.6	5.4 ± 0.51	5.5 ± 0.52	5.2 ± 0.49	5.2 ± 0.49
	Bison	389	1931 May 16	4.7 ± 0.22	4.1 ± 0.19	3.9 ± 0.18	3.8 ± 0.17
	Damont	3	May 16	4.6 ± 0.38	4.2 ± 0.35	4.2 ± 0.35	4.3 ± 0.36
Langdon, N. Dak.	Bison	389	1931 May 14	15.0 ± 1.51	11.5 ± 1.16	16.8 ± 1.70	16.8 ± 1.70
	Damont	3	May 14	11.4 ± 0.98	13.0 ± 1.12	12.4 ± 1.07	15.9 ± 1.37
	Bison	389	1932 May 10	14.9 ± 0.41	13.8 ± 0.38	13.6 ± 0.37	14.7 ± 0.40
	Do	389	May 25	12.7 ± 0.90	9.7 ± 0.69	15.5 ± 1.10	12.7 ± 0.90
	Do	389	June 10	3.8 ± 0.48	1.9 ± 0.24	3.7 ± 0.46	2.9 ± 0.36
	Do	389	1933 May 5	4.0 ± 0.54	6.6 ± 0.89	3.7 ± 0.50
	Do	389	May 19	6.2 ± 0.71	6.1 ± 0.70	6.4 ± 0.74
	Do	389	June 15	4.7 ± 0.56	3.9 ± 0.47	3.7 ± 0.45

TABLE 1.—(Continued).

Station	Variety	C. I. no.	Date sown	Stand in plants per rod row				Yield in bushels per acre			
				Non-treated check	Treated with			Non-treated	Treated with		
					Formalde- hyde spray	Ceresan dust	Copper carbonate dust		Formalde- hyde spray	Ceresan dust	Copper carbonate dust
Mandan, N. Dak.	Bison	389	1931	12.3 ± 0.33	13.0 ± 0.35	13.4 ± 0.36	13.4 ± 0.36
	Damont	3	1931	12.0 ± 0.76	12.7 ± 0.80	10.9 ± 0.69	13.1 ± 0.83
	Bison	389	1932
	Do	389	May 2	q1670 ± 123.3	q715 ± 52.8	q1466 ± 108.3	q1521 ± 112.6	17.0 ± 0.54	14.1 ± 0.45	16.7 ± 0.53	16.2 ± 0.52
	Do	389	May 16	q1212 ± 40.0	q965 ± 31.8	q1183 ± 39.0	q1053 ± 34.8	12.6 ± 0.29	12.2 ± 0.28	12.4 ± 0.29	11.7 ± 0.37
	Do	389	June 3	q1143 ± 33.2	q548 ± 15.9	q1183 ± 34.4	q1016 ± 29.4	9.4 ± 0.28	8.1 ± 0.24	9.5 ± 0.28	9.5 ± 0.28
Morris, Minn.	Do	389	1933	2.9 ± 0.16	3.2 ± 0.17	3.3 ± 0.18
	Do	389	May 2	q1533 ± 43.6	q1535 ± 43.7	q1598 ± 45.4	2.8 ± 0.09	2.8 ± 0.09	2.8 ± 0.09
	Do	389	May 16	q1623 ± 71.5	q1577 ± 69.4	q1503 ± 66.2	0.8 ± 0.22	0.8 ± 0.22	0.8 ± 0.22
	Do	389	June 1	q999 ± 42.0	q1141 ± 47.9	q1064 ± 44.7	6.1 ± 0.94	5.7 ± 0.88	6.4 ± 0.99	6.1 ± 0.94
	Bison	389	1931	1.6 ± 0.40	0.9 ± 0.22	1.7 ± 0.43	1.5 ± 0.38
	Damont	3	1931	4.4 ± 0.40	4.1 ± 0.37	4.4 ± 0.40	4.1 ± 0.37
St. Paul, Minn. (farm soil)	Bison	389	1931	285 ± 14.5	255 ± 13.0	318 ± 16.2	258 ± 13.2	0.9 ± 0.15	0.8 ± 0.13	1.3 ± 0.21	1.1 ± 0.18
	Damont	3	1931	50 ± 6.8	50 ± 6.8	71 ± 9.7	69 ± 9.4	5.3 ± 0.22	6.2 ± 0.25	5.3 ± 0.22	4.8 ± 0.20
	Bison	389	1931	310 ± 20.7	341 ± 22.8	319 ± 21.3	309 ± 20.6
St. Paul, Minn. (wilt-sick soil)	Damont ^e	3	1931	12.2 ± 0.80	5.8 ± 0.38	14.3 ± 0.94	13.4 ± 0.89
	Bison	389	1932	453 ± 31.2	151 ± 10.4	629 ± 43.4	623 ± 42.9
	Bison	389	May 3	497 ± 19.9	199 ± 8.0	557 ± 22.3	604 ± 24.2	7.6 ± 0.23	8.9 ± 0.27	8.7 ± 0.27
Waseca, Minn.	Bison	389	1933	456 ± 9.6	645 ± 13.5	655 ± 13.7	2.6 ± 0.27	2.2 ± 0.23	2.1 ± 0.22
	Bison	389	May 6
	Bison	389	May 16
	Bison	389	1932	176 ± 7.6	39 ± 1.7	311 ± 13.3	239 ± 10.3

^a Yield from 8 foot row.^b Number of plants in 4 feet of row.^c Completely killed by wilt.^d No. of plants per A. (000 omitted).^e Adverse climatic conditions destroyed the crop from this and later sowings.

yields were equal. In 27 tests, the plots sown with Ceresan-treated seed yielded more than those sown with seed treated with copper carbonate; in 21 tests, they yielded less; and in 7 tests the yields were identical.

TABLE 2.—*Summary of the statistical analysis of the results of flax seed-treatment tests at 11 stations in Minnesota, North Dakota, South Dakota, and Montana*

		Number of tests in which statistically significant differences in stand or yield were obtained between the treatments compared											
No. of tests in which treatments were compared	No. of tests in which there was no significant difference between any of the treatments	Check better than formaldehyde	Check better than Ceresan	Check better than copper carbonate	Formaldehyde better than check	Formaldehyde better than Ceresan	Formaldehyde better than copper carbonate	Ceresan better than check	Ceresan better than formaldehyde	Ceresan better than copper carbonate	Copper carbonate better than check	Copper carbonate better than formaldehyde	Copper carbonate better than Ceresan
Stand													
24	6	15	0	0	0	17	15
28	9	0	2	3	3	4	0
Yield													
47	31	9	0	0	1	11	9
55	38	0	1	1	1	1	1

The statistical analysis of the results at all cooperating stations, a summary of which is given in table 2, brings out more clearly the variability of the results and the general failure of the treatments to benefit either stand or yield. The formaldehyde treatment was included in 18 of the 19 tests in which significant differences in stand were obtained. In 17 of these 18 tests, the stand from the Ceresan-treated seed, and in 15 tests each, the stand from the copper carbonate-treated and the non-treated check seed was significantly greater than that from the seed receiving the formaldehyde treatment. The 4 tests in which either or both Ceresan and copper carbonate dust treatments gave significantly better stands than the non-treated check were at St. Paul and Waseca, Minn., stations at the edge of the present seed-flax producing region.

The wilt-susceptible variety, Damont, was included only in the 1931 tests. It was sown on farm soil at all the cooperating stations, and at St. Paul, Minn., and Fargo, N. Dak.; additional sowings were made on wilt-infested soil. No treatment had a noticeable effect on the occurrence of wilt in this variety and no significant differences in yield were obtained. Stand counts

were made only at St. Paul but there were no significant differences between the treatments.

The effect of the different seed treatments on yield resembled that on stand, except that injury due to the formaldehyde treatment was less pronounced. In 38 of the 55 tests in which yield data were obtained, there were no significant differences in yields between any of the treatments. In only 1 of the 55 tests were the yields from plots sown with treated seed significantly greater than the yield from those sown with non-treated seed. This was the first date-of-sowing test on farm soil at Fargo, N. Dak., in 1933, in which the probable error was small, and the plots sown with seed treated with Ceresan and copper carbonate yielded more than those sown with non-treated seed by significant amounts. One instance in which the non-treated seed yielded more than that treated with copper carbonate, one in which the Ceresan-treated yielded more than the copper carbonate-treated, one in which the copper carbonate-treated yielded more than the Ceresan-treated, and one in which the formaldehyde-treated yielded more than the copper carbonate-treated by significant amounts were also recorded. The formaldehyde treatment was included in 47 of the tests from which yield data were obtained and in 16 of these there were significant differences in yield between the various treatments. In these 16 tests, the yield from the formaldehyde-treated seed was significantly inferior to that from the seed treated with Ceresan, in 11 cases, to that treated with copper carbonate, in 9 cases, as well as to the yield from the untreated seed, in 9 cases.

DISCUSSION

Although drought conditions were more or less general during the 4-year period covered by these tests, yields averaging more than 10 bushels per acre were obtained in approximately half of the tests from which yield data were secured. Consequently, it is believed that the general failure of the seed treatments to benefit either stand or yield cannot be attributed entirely to the occurrence of weather conditions unfavorable for flax production. European investigators (3, 6) have noted that seed treatment was most beneficial when seed of inferior quality was used, and in seasons when seedling-blight and damping-off (*Phythium* sp. and *Rhizoctonia* sp.) were prevalent. However, Burnett and Reddy (2) did not report any observation on these seedling diseases in tests in which they secured beneficial effects from the Ceresan dust treatment. The seed used in the tests reported in this paper was of good quality and no reports of seedling-blight or damping-off were received from any of the collaborators. Several of the collaborators noted a delay in germination and poorer stands in the plots sown with formaldehyde-treated seed, but no reports were received of any stimulatory effect of the Ceresan or copper carbonate treatments.

It should be pointed out that the only tests in which the stand from treated seed was significantly better than that from untreated seed were those conducted at St. Paul, Minn., in 1932 and 1933, and at Waseca, Minn., in 1932. These stations, as well as those in Iowa at which Burnett and Reddy (2) secured such beneficial effects from the Ceresan treatment, are in regions that have been under cultivation longer and have a more humid climate than those in which the other stations cooperating in these tests were located. The more humid conditions and the longer period that the soil has been under cultivation would tend towards an increase of the damping-off organisms in the soil. Although neither damping off nor seedling-blight were reported either in Minnesota or in the tests conducted by Burnett and Reddy in Iowa, it is possible that they escaped detection, as the plots were not under continuous observation. Flax is most susceptible to injury by the damping-off and seedling-blight organisms while very young, often failing to emerge, and when emergence of attacked plants does take place, the latter are often rapidly killed. The killed plants soon dry up and in a few days their detection is difficult.

It seems probable that seed disinfection would produce beneficial results in cases where the seed was contaminated with such seed-borne organisms as those causing canker or seedling-blight and browning. It is even possible that the residual effect of the fungicide on the seed coat might tend to delay the attack of the damping-off fungi. In regions where flax culture has been recently initiated and seed-borne diseases are not yet established, proper seed disinfection should aid in preventing or at least in delaying the establishment of these diseases. However, the tests reported in this paper indicate that little benefit can be expected from treating flax seed in North Dakota, South Dakota, Montana, and western Minnesota, and that under certain conditions the formaldehyde-spray treatment may be injurious.

SUMMARY

The results of cooperative flax seed-treatment tests at 11 stations in Minnesota, North Dakota, South Dakota, and Montana, are summarized.

The object of these tests was to determine the comparative effectiveness of the formaldehyde-spray and the Ceresan and copper carbonate dust treatments in controlling diseases and in increasing stand and yield of flax.

Flax diseases amenable to seed treatment, such as seedling-blight, browning, and damping-off, were of negligible importance during the four years covered by these tests and no accurate information was secured concerning the effectiveness of the different seed treatments in controlling these diseases.

None of the treatments had an appreciable effect on the incidence of wilt, in the wilt-susceptible variety, Damont, in 1 year's trials on either wilt-infested or non-infested farm soils.

None of the treatments had a consistently stimulatory effect on either stand or yield. There was some indication that, under certain conditions, Ceresan and copper carbonate dust treatments benefited the stand in the more humid region of southeastern Minnesota.

The formaldehyde-spray treatment was distinctly injurious under conditions that prevailed during the period of some of these tests. It caused significant reductions in stand in approximately three-fourths of the tests and in yield in approximately one-third of the tests in which such data were secured.

Treatment of flax seed does not appear to be justified at present in the seed-flax producing regions of North Dakota, South Dakota, Montana, or the western part of Minnesota.

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THE INFLUENCE OF DRY AIR ON THE LONGEVITY OF THE FIRE-BLIGHT PATHOGEN¹

H. R. ROSEN

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It is commonly believed that the fire-blight pathogen, *Erwinia amylovora* (Burr.) Com. S. A. B. (*Bacillus amylovorus*) is very sensitive to dry air.

"The pear-blight microbe is a very delicate organism and cannot withstand drying for any length of time. In blighted twigs exposed to ordinary weather it dries out in a week or two and dies" (17).

"This germ forms no spores, is very sensitive to drying, and, in fact, is a very short-lived organism. It dies rapidly in the blighted tissues as soon as they have become fully killed by the germs. It cannot withstand drying, usually dying within two weeks to a month" (18).

"The process of killing (referring to the fire-blight pathogen) is often, although not always, simply that of desiccation" (3).

The fire-blight organism is "dry-air-sensitive" (p. 369) and is (p. 380) "killed quickly" by dry air (13).

"It has been demonstrated that the bacteria will live several weeks, or even months, in the blight-cankered limbs that have been cut away and left lying around. As these dry out, however, the bacteria die with the dying wood" (4).

"The more concentrated sugar solutions (such as are found in blossoms in dry weather) not only are unfavorable to the development of bacteria, but also weaken their virulence so that they are feeble or entirely impotent in the production of blight" (16).

Contrary to these citations and various others of similar nature, there are a few investigators who have found the fire-blight pathogen to be relatively long lived under dry conditions. Among these are Waters (19) who very briefly noted that in New Zealand the bacteria under consideration are "capable of living in dried exudate for a period of nine months." How "dry" the environmental conditions were, is not stated. Similarly, Thomas, as reported by Pierstorff (8), "obtained virulent organisms twelve months after infected limbs had been brought into his laboratory," and Pierstorff (8) obtained pure cultures of the organism for a period of 2½ years from dried ooze on Transcendent crab-apple trees, kept in the laboratory.

So far as these empirical tests are concerned, and similar ones made by the writer, there is no clear understanding as to the actual "dryness" of the

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ooze or of the material from which the bacteria were cultured. Likewise, the common procedure of smearing pure cultures of bacteria on sterilized glass slides or cover slips that are allowed to remain "dry" under diverse laboratory conditions for varying lengths of time, gives neither a clear idea of the actual dryness involved nor of the possible behavior of the bacteria on natural hosts (9, p. 381). Aside from daily and seasonal fluctuations in air humidity in any one region, there is of course much difference to be expected in this respect in different parts of this country as well as in unlike countries, and what would be considered dry in one region might well be taken to be otherwise in another. Clearly, unless one knew the relative humidity or saturation deficit, or had some other measure of the dryness to which any given material is subjected, there can be no adequate means of duplicating comparable conditions or of drawing conclusions relative to the response of *Erwinia amylovora* to actual dry conditions.

EXPERIMENTAL DATA

For a comparison with results obtained in other sections of the country, it was considered desirable in preliminary experiments to attempt to duplicate in some measure the previous work. For this purpose, hardened bacterial droplets of exudate attached to blighted tissues and kept in ordinary paper packets in the laboratory for varying lengths of time, were on May 24, 1934, placed in small quantities of sterile water and allowed to form a noticeable cloudy suspension. Several hypodermic injections were made from each collection into tender Bartlett pear shoots maintained on potted plants in a greenhouse. Exudate from the following material was used:

1. Jonathan apple twig gathered April 15, 1927.
2. Bartlett pear twig gathered April 22, 1927.
3. Jonathan apple twig gathered April 29, 1927.
4. Jonathan apple twig gathered May 3, 1927.
5. Jonathan apple twig gathered May 8, 1933.
6. Jonathan apple twig gathered May 5, 1934.

It will be noted that in the first 4 of these, the exudate was about 7 years old; in the 5th one, slightly more than a year old; and the last, to serve as a check, not more than 19 days old. As a further check on the injections, a series of poured dilution plates with nutrient dextrose agar was used from one of the older samples of exudate (number 1). The droplets of hardened ooze varied in size from that of a mustard seed to that of a garden pea. The results were as follows: No infections were obtained and no bacterial colonies resembling *Erwinia amylovora* developed from the first 5 samples. All the shoots inoculated with Sample 6 produced typical blight symptoms within 4 days after the inoculations.

On June 6, 1934, the experiment was duplicated, except that Sample 1 was not represented. Likewise, a series of poured dilution plates was made from each sample. The results were similar to those previously ob-

tained. Only from Sample 6, representing exudate from the current year, were infections produced and colonies of *Erwinia amylovora* obtained.

One may conclude either that Thomas and Pierstorff's findings in New York are not applicable to Arkansas conditions with reference to the longevity of bacterial exudate kept under laboratory conditions, or that other factors than dryness may be involved. While it is not surprising to find material 7 years old failing to yield virulent cultures, it is obvious that the one-year-old material should have produced infections, if Thomas's and Pierstorff's findings are correct and widely applicable.

But as stated previously, the "dryness" of an Ithaca, New York, laboratory may not be comparable to that of one in Fayetteville, Arkansas. Consequently, a more exact experiment was designed to answer the question relative to the influence of dry air on fire-blight bacteria contained in hardened exudate. It consisted of, first, the use of concentrated sulphuric acid in a sealed, glass bottle with bacterial exudate suspended over the acid;² second, as a control, bacterial exudate gathered at the same time and on the same host placed in corked vials outdoors without acid; and third, as a further check, bacterial exudate was permitted to remain in place on a blighted pear tree.

The method used for placing the exudate over the sulphuric acid was essentially the same, with slight modifications, as that used by Noble (5). One modification consisted in using about 100 c.c. of c.p. acid, with a specific gravity of 1.835 without any water added, in a 200 c.c. large-mouth bottle, and suspending a paraffin-covered paper basket from the rubber stopper. The bottom of this open basket rested slightly less than one inch above the level of the acid. The exudate, gathered from blighted Jonathan apple and Bartlett pear shoots on May 5, 1934, and on June 11, 1934, was kept in the laboratory with the bases of the shoots immersed in water until June 12, 1934. On that date the exudate was removed from the diseased host tissues and divided into 2 lots. One lot was placed in the paper basket suspended over sulphuric acid, the bottle being tightly stoppered and sealed with melted paraffin, and the other lot was placed in small vials, corked but not sealed with paraffin, and hung in a wire basket suspended from a limb in an apple tree.

The exudate, which was permitted to remain in place on a blighted pear trunk, consisted of a thick copious flow that had occurred on the east side of the trunk from a canker of the current year's origin. It was first observed on June 23, and judging by the color and hardness of the ooze, it must have exuded approximately a week or 10 days previously. That it was

² The avidity of this acid for water has been made use of by previous investigators in attempting to get measurable degrees of relative humidity for determining the behavior of various species of fungi and bacteria under varying degrees of dryness (15, 6, 7, 5).

still in place and rather sharply delimited, indicated that it appeared after the heavy rain, wind, and hail storm of June 10. This date marked the beginning of a dry period that remained unbroken until the latter part of August. This extreme drought, shared by a large part of the country, enabled the gathering of portions of the pear exudate throughout the summer and formed a desirable check on the exudate suspended over the concentrated sulphuric acid. The portion of the trunk upon which this exudate appeared was fully exposed to the east sunlight.

The method of testing and the results of the tests made with these 3 different treatments of exudate are as follows: The exudate in each instance was placed in sterile water, the bacteria permitted to form a heavy suspension, and used both as direct injections into healthy succulent shoots of Bartlett pear in the greenhouse, and for poured dilution plates with nutrient dextrose agar as the medium. Samples from the exudate left in place on the blighted pear trunk were gathered on June 26 and on July 17 and tested for viability and infectivity. The results were wholly negative, no

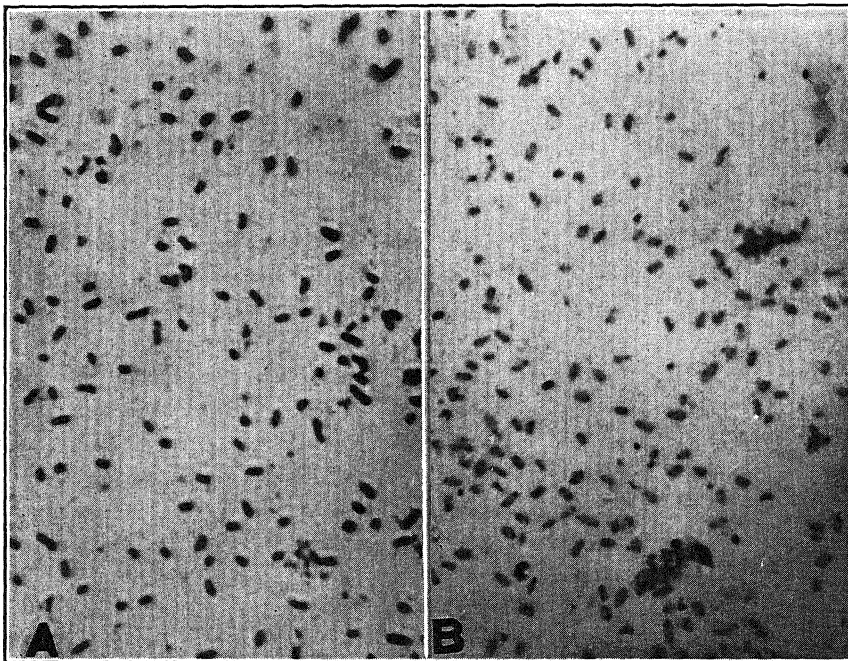


FIG. 1. Fire-blight bacteria contained in exudate ($\times 1840$). A. Kept at a relative humidity close to zero for a period of about 8 months. These bacteria were viable and virulent. B. Kept in closed, humid vials outdoors for about 8 months, when they were no longer viable. Note how faintly and unevenly the bacteria are stained with polar granules often in evidence.

colony appearing on the poured plates that resembled *Erwinia amylovora*, and no infections appeared from the direct injections with the diluted exudate.

Samples from the exudate placed in corked vials and suspended from an apple tree were gathered in September, November, and December and similarly tested. Here again the results were negative, and when a stained smear of the exudate made early in January, 1935, was compared with the still viable and infectious exudate kept over sulphuric acid, the bacteria were largely in the form of relatively minute ghost cells, often containing faintly stained polar granules (Fig. 1, B). It was noted that the vials contained free moisture, and that the exudate was covered with various molds within several weeks after they had been placed in the vials.

Samples from the exudate placed over concentrated sulphuric acid were tested on September 10 and November 12, 1934, and March 19, and May 7, 1935. The last date represented a full year after the first exudate had been gathered. In each instance viable and infectious bacteria, producing typical fire-blight symptoms, were obtained. The specific gravity of the sulphuric acid was tested on November 12, 1934, and again on May 7, 1935, and gave in both instances a reading of 1.8140, due allowance being made for temperature.

The fact that *Erwinia amylovora* in the form of exudate can live for a year at least (the tests are being continued) in a bone-dry atmosphere in which the relative humidity is close to zero,³ constitutes a wholly unexpected phenomenon that is not at all in accord with prevailing belief concerning the influence of dryness on the longevity of fire-blight bacteria. Obviously, some other factors are involved in reducing the length of life of these bacteria when in the form of extruded ooze under some outdoor conditions or within various host tissues on the plant. The nature of these factors can merely be conjectured, but among those that must be considered are sunlight, other microorganisms, and the chemico-physical composition of the host materials in contact with the bacteria. Instead of dryness being the cause of short life, it may be that the reverse is true. The presence of various quantities of moisture would result not only in changes in the physical condition of the bacterial protoplasm and of the gelatinous matrix in which the bacteria are enveloped, but also in favoring the germination and growth of air- and water-borne competing microorganisms. The writer has already directed attention to a yellow bacterial saprophyte rather commonly associated with *Erw. amylovora* that has a marked inhibiting effect on the life of the fire-blight bacteria (10, p. 65).

³ According to the physical tables (15, 20, 21), concentrated sulphuric acid with a specific gravity of 1.8140 yields a relative humidity of around zero.

Aside from the studies detailed above, 2 additional lines of investigation have been in progress during the past few years on the exudates of fire blight. While these were conducted with the primary purpose of determining the presence or absence of live bacteria on the surface of blighted and overwintered tissue and in the soil beneath badly blighted pear and apple trees, they have a direct bearing on the question under discussion. As previously reported (11, p. 50), in approximately 150 different attempts to isolate the pathogen from the surface of diseased twigs and limbs of both apple and pear, gathered throughout the winter and early spring of 1933-1934, all were negative. Only 2 positive isolations have been obtained, both in midwinter of 1932-33, one by soaking a blighted apple twig in sterile water for a few minutes, and the other by first coating the cut end of a blighted pear twig with paraffin and then soaking. In view of the very small number of successful isolations, it may be questioned whether bacterial exudate remaining on the exposed surfaces of twigs and limbs of pear or apple forms a common or important means of overwintering. In view of the findings here reported on the influence of dry air on fire-blight bacteria, this is to be expected, since dryness for any length of time during the fall and winter is seldom experienced in the fruit-growing section of Arkansas. On the other hand, in exceptional dry seasons or in dry shelters during moist seasons, and when other factors would not counterbalance the aridity, or in any region that normally has a very dry climate, it is conceivable, in view of the results here reported, that the bacteria would have a better chance of surviving for greater time intervals.

Much of the bacterial ooze extruded from pear and apple tissues must undoubtedly reach the soil beneath and around the trees, and also possibly be carried at times by strong winds when accompanied by rains into upper air currents and be deposited considerable distances from the place of origin. Ark (1) has found that in artificially contaminated sterilized soils the organism will live as long as 54 days at a temperature of 8° C., 30 days at 21°, and as long as 38 days in non-sterilized soil kept at 8° and at 21°. From soils naturally contaminated he obtained infections from samples gathered through the summer and up to November 18. Thomas and Ark (16) in additional studies found the bacteria viable in naturally contaminated orchard soil throughout the winter of 1932-33 up to March 31, but the number of successful isolations gradually diminished. They conclude, therefore, that their results suggest that the soil is probably unimportant in overwintering the bacteria.

During the fall and winter of 1934-35, the soil from beneath badly blighted pear and apple trees, from one orchard at Fayetteville and another near Rogers (Benton County), Arkansas, was investigated for the presence of infectious fire-blight bacteria. In each instance the sample was charted

with reference to location, the trowel flamed after each sample was gathered, and the soil placed in sterilized cardboard containers, weighed shortly after it was collected. Only the top 2 inches was utilized. The weights varied in the different samples from about 200 to 300 grams, the average being around 250 grams (moist weight). Each sample was placed in 100 c.c. of sterile water and a series of dilution plates made from each. The diluted soil mixture also was used for direct hypodermic injections into Bartlett pear shoots. The results are as follows: From 15 soil samples gathered near Rogers on December 19, 1934, none yielded *Erwinia amylovora*. Likewise, from 42 soil samples gathered at Fayetteville on January 14, February 16, and March 1, 3, and 9, 1935, no fire-blight bacteria were obtained.

These negative results, however, are not considered conclusive, for the reason that the technics now available for soil isolations of *Erwinia amylovora* are far from being certain. The writer has found that the crystal-violet-bile agar medium (1), which tends to eliminate gram-positive microorganisms, also has a more or less marked deleterious effect on the growth of the fire-blight pathogen. This effect, it was found, varied considerably and depended in large part on the batch of dye in use. Apparently, crystal violet has been insufficiently standardized to yield the same results at all times. The samples of this dye at hand, when used as Ark (1) recommended, in dilutions up to 1 to 100,000 on pure cultures of *Erw. amylovora*, and compared with similar quantities of inoculum on nutrient dextrose agar, inhibited approximately 75 per cent of the colonies. Furthermore, when a soil sample is diluted with water, and then inoculated into susceptible hosts, there is no assurance that infections will be obtained, even if *Erw. amylovora* is present. As will be reported elsewhere, the degree of bacterial dilution, other things being equal, has a very direct bearing on the initiation and development of blight.

Although one may properly question the reliability of the tests utilized on soil samples for determining the presence of *Erwinia amylovora*, the results cited previously concerning the influence of dry air on bacterial exudate, suggest that the longevity of the fire-blight pathogen in the soil would depend in part at least on the degree of dryness of the soil. In moist or wet soil, the bacteria, in accordance with these tests, would not live so long as in dry soil. Likewise, in dry soil, unless the bacteria become lodged in places that shield them from the direct rays of the sun, they would be markedly inhibited.

The results obtained with the exudate exposed to sunlight on a pear tree also suggest that, under such conditions, the bacteria die in short order, not because of the dryness but probably because of the germicidal action of the sun's rays. The effect of different temperatures in the presence of dry air

must also have its influence on the longevity of the bacteria, but this remains to be determined.

It is to be especially noted that the experiments detailed above were confined wholly to exudates extruded naturally from blighted host tissues. If pure cultures from artificial media were used, the results might be quite different. Peltier (6), who made a thorough painstaking study on the effect of temperature and humidity on the citrus-canker pathogen, *Phytopomonas citri*, found that when pure cultures of the citrus-canker bacteria, grown in nutrient broth, were used for humidity and temperature studies, humidity had little or no influence at low temperatures, while at high temperatures, high humidities were the limiting factors. At medium humidities the organism was viable at all temperatures for the period of the experiment (8 days). Working with the same pathogen, Stevens (14) reported that it was viable and infectious when kept in dry sterilized soil for a period of 26 months.

THE INFLUENCE OF DRY AIR ON THE MORPHOLOGY OF THE ORGANISM

It has been suggested (8) that there may be resting forms of *Erwinia amylovora*, as cysts or involution forms "which may be more resistant to adverse conditions and may approximate in function and characteristics the endospores formed by such species as *Bacillus botulinus*."

To test this theory a microscopic study was made of stained and unstained preparations of bacterial smears from the viable exudate kept over sulphuric acid, as well as from the nonviable exudate kept in vials outdoors. The most decided differences noted in both the stained and unstained preparations was the relative minuteness in size and the relative emptiness of the nonviable bacteria. They appeared to be mostly empty shrunken shells, with granules appearing frequently near the poles. In contrast to these, the viable bacteria were individually one to several times as large as the nonviable ones, and stained evenly and heavily with carbol-fuchsin (Fig. 1, A). There was no suggestion whatever of involution forms or cysts or endospores. When the bacteria kept over sulphuric acid for approximately 12 months were compared in stained smears with the bacteria contained in fresh exudate, there was no appreciable differences noted in form, in size, or in staining reaction.

When the bacteria that had been suspended over sulphuric acid for 8 months were compared with those in corked vials, kept outdoors for a similar length of time, using the Gay and Clark (2) recommendation (Proca-Kayser stain) for the differentiation of living from dead bacteria by staining reactions, the living bacteria kept in the very dry air stained violet or violet red, while the dead ones contained in the vials appeared as clear red. This differentiation was not quite so clear as Gay and Clark have

found for other bacteria, in which living ones stain blue and the dead ones red by the method utilized.

DISCUSSION

While the results here presented leave no doubt that the fire-blight producer can withstand exceedingly arid conditions for relatively long periods under indoor conditions, it still remains to be shown that it will do likewise outdoors under natural conditions. So far as the indoor temperatures are concerned, the laboratory temperatures during the year in which the bacteria were confined in the sulphuric acid bottle varied from 35° C. in the extreme heat of the summer of 1934 to around 20° C. during the winter. Naturally, the fluctuations were not so sharp as occurred outdoors. Nevertheless, if any exudate happens to become lodged in rifts or bark cracks or in any other place in and around the orchard, which may prevent access of rain or of direct sunlight, it is quite conceivable that they would remain alive for a relatively long time.

It seems desirable to point out that the studies here reported are confined entirely to the viability and infectivity of the organism. It does not concern its growth and reproduction. While dry conditions are seemingly conducive to long life, this does not mean that such conditions are also conducive to infection and disease development. Indeed, Shaw (12) has shown quite conclusively that a high relative humidity is necessary for good growth of *Erwinia amylovora*, 99.9 per cent relative humidity yielding maximum growth and grading downward to almost no growth at 95 per cent. Likewise, within susceptible hosts there is a direct relation between increased fire-blight resistance and lowered relative humidity in the intercellular spaces; 97 per cent relative humidity in these spaces was associated with complete resistance to infection and 100 per cent with maximum susceptibility. Shaw's studies, coupled with those here reported, serve as an excellent illustration of an apparently common phenomenon of microscopic parasites, namely, conditions favorable for long life are often the reverse of those that favor growth and pathogenic expressions.

SUMMARY

In air-dry bacterial exudate attached to blighted twigs and exposed to laboratory air at Fayetteville, Arkansas, for varying periods of time, the bacteria were found to be dead at the end of a year.

When similar exudate is kept under the same conditions, but is otherwise confined in an atmosphere approaching a relative humidity of zero, the bacteria are still viable and infectious about a year after they have been confined to these extremely dry conditions.

Fire-blight exudate kept in corked vials suspended from an apple limb under outdoor conditions yielded no viable fire-blight bacteria within 3 months after the exudate was thus confined.

Exudate left in place on a cankered pear trunk and gathered at various intervals after it had extruded from the tissues, showed no viable pathogens within 3 weeks after its extrusion. This exudate was fully exposed to direct sunlight, but remained dry during the summer drought of 1934.

Tests made from 67 soil samples taken beneath badly blighted apple and pear trees during the winter months of 1934-35, from December to March, showed no viable *Erwinia amylovora* in any exudate that may have lodged in the soil. These tests, however, owing to the questionable technics now available for isolating the fire-blight pathogen from nonsterilized soil samples, are not considered conclusive evidence for absence of this parasite in the soil during the winter months. The experiments cited previously suggest that, under soil conditions, the longevity of the organism would depend in part on the degree of dryness of the soil, and this varies from year to year.

A morphological study of the viable bacteria kept under extremely dry conditions for about a year show no endospores, cysts, or involution forms. When compared with bacteria kept outdoors in closed vials, under conditions in which they have lost viability, the dry living bacteria are larger and stain more heavily and evenly than the dead ones. The Proca-Kayser method for the differentiation of living from dead bacteria by staining reactions stains the viable bacteria violet or violet-red and the dead ones clear red.

AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARK.

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SPREADER MATERIALS FOR INSOLUBLE COPPER SPRAYS

H. C. YOUNG and J. R. BECKENBACH

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Many and varying types of spreaders¹ are being used with spray materials. They are placed there because of their effect on spreading, sticking, and fluffing; as diluents, absorbing agents, and stabilizers; and for protection to plants and, perhaps, for many other purposes. In many cases a spreader material may be satisfactory in one way and of little use as a general corrective agent. There are many spray materials that require reinforcement only in spreading or adhesiveness; there are other materials requiring a diluent, such as many of the dust mixtures; and there are others that are active as fungicides or insecticides when they are in a soluble form or in the gaseous state. Such materials, to be effective and lasting, must necessarily be adsorbed by a spreader that has most of the above qualifications.

No attempt will be made here to review the literature dealing with spreader materials. The greater part of the investigations on this subject has been done by concerns manufacturing spray materials who have either kept their results secret or have patented their combinations. In addition, many proprietary spreaders have appeared on the market. As a result of the writers' attempt to review the literature it was found that this or that material had been used, but rarely were comparisons made or fundamental reasons given for its particular use. The problem of spreaders for spray materials is complicated and too little fundamental research has been done on it.

This investigation has had to do primarily with the clay type of spreaders. For the past several years considerable experimental work has been done on a number of insoluble copper compounds. These coppers have been developed as substitutes for Bordeaux mixture in an attempt to reduce copper injury. Their effectiveness as fungicides has been somewhat disappointing, and preliminary tests indicated that a part of this ineffectiveness was due to their lack of adhesiveness. An attempt, therefore, was made to add a spreader material in a ratio that would correct this defect. Since it was necessary, too, to select a material that was chemically inactive, the safest group seemed to be the clays.

The method used in this work was the thorough mixing of the insoluble copper with the spreader material and then the atomizing of the mixture onto glass slides. In preliminary tests it was found that paraffining, shellacking, or painting the slides did not add to the adhesiveness. It also was

¹ Used as a general term.

found that clean glass slides were very comparable to foliage. The various combinations were mixed in a mortar and made up to the correct proportions, the formula used being comparable to those generally followed in making up sprays. The mixtures were then atomized onto thoroughly cleaned glass slides with a No. 15 DeVilbiss atomizer. The slides were $3\frac{1}{4}$ by 4 inches; the atomizer was held 22 inches from the slide, and 16 full squirts in rapid succession were applied to each slide. This gave a complete coverage of fine drops and was very comparable to sprayed leaves. Each slide was allowed to dry and then was dipped 10 times in 80 c.c. of distilled water. Each dip included the complete immersing of a slide, raising it from the container, draining it, and giving it a sharp jerk so that the large drops of water fell into the beaker. The alternate dipping and draining were of approximately 1 second duration each. The wash water was then transferred to a 100 c.c. volumetric flask and made up to 100 c.c. with 1:2 hydrochloric acid.

The residue on the slides was removed with 75 c.c. of 1:5 hydrochloric acid made up to 100 c.c. The sodium diethyl dithiocarbamate colorimetric quantitative method of Williams² was used with necessary modifications.

The necessary aliquot, containing the amount of copper within reading range of the standard solution³ was placed in a small separation funnel and 10 c.c. of 1 per cent sodium diethyl dithiocarbamate solution added.

Color was extracted with 10 c.c. of chloroform by shaking vigorously in the separation funnel. This was then compared with the standard in the colorimeter, the reading noted, and computations made.⁴

The spreader materials, copper compounds, formulae and results are given in table 1. The results are averages of duplicates.

The results indicate that basic copper sulphate, basic copper chloride, and copper phosphate, when used without a sticker, adhere to slides very poorly. Of the clays used, bentonite and a specially treated bentonite called Wyojel were effective. These 2 bentonites also varied in their effect on sticking when larger percentages were used.

In order to determine the effect of the quantity of bentonite and Wyojel on sticking a second series was arranged. The amount of copper was held constant in the formula and the amounts of the 2 clays were varied from

² Williams, W. The determination of copper and iron in dairy products. Jour. Dairy Research 3: 93-100. 1931.

³ The copper standard contains .02 mg. copper, using C.P. CuSO_4 . Computations made on copper content. The standard is made up by taking an aliquot containing .02 mg. copper, adding 10 c.c. of .1% carbamate solution, a few drops of HCl, and extracting the color with 10 c.c. chloroform.

⁴ Computations from colorimeter reading. Sample determination.

$$\frac{25 \text{ (Set reading of standard on colorimeter)}}{24.2 \text{ (Reading of unknown)}} \times .02 \text{ (mg. copper in standard)} = \frac{25}{24.2} \times .02 = .02066 \text{ mg. of copper in unknown.}$$

TABLE 1.—Copper residues of basic copper sulphate, basic copper chloride, and copper phosphate on slides after washing

Spreader material	Per cent copper residue 2-2-50 formulab			Per cent copper residue 2-10-50 formulab		
	Basic copper sulphate	Basic copper chloride	Copper phosphate	Basic copper sulphate	Basic copper chloride	Copper phosphate
Check—no spreader	33.8	27.2	22.6
Bentonitea	95.5	89.6	92.7	59.6	26.6	32.0
Wyojel	95.0	92.3	90.5	85.4	96.4	54.7
Talc	38.8	62.3
Bancroft clay	41.6	74.0
Flour	60.6
Bordeaux, 4-6-50	97.0

a Wyobond bentonite was used in all of these tests.

b Pounds in gallons of water.

0.1 pound to 10 pounds. The formulae and results are given in table 2 and are shown graphically in figure 1.

The physical difference between the bentonites and the other clays, such as kaolin, talc, and Bancroft, was in the flocculation. This is shown in figure 2.

The bentonite and Wyojel flocculated about the same as Bordeaux mixture, but settled slightly more rapidly. The other clays did not flocculate, and they settled out with the copper in a very few minutes. It was first thought that there was a definite correlation between flocculation and sticking, since the effect of the bentonites was much greater than in the case of

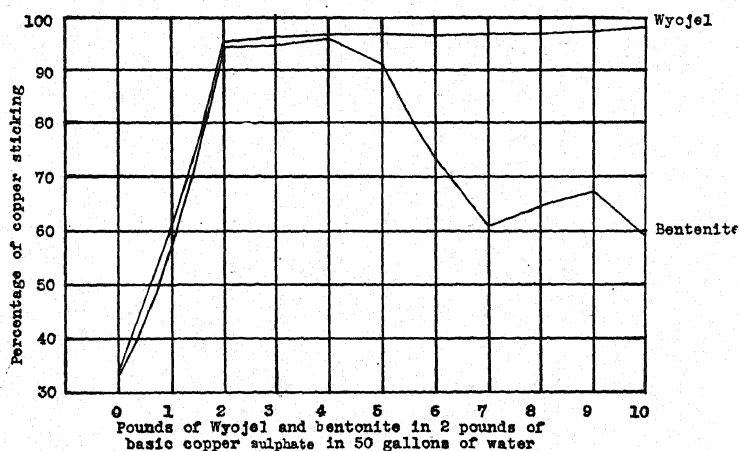


FIG. 1. Comparative effect of Wyojel and bentonite on adhesiveness of basic copper sulphate.

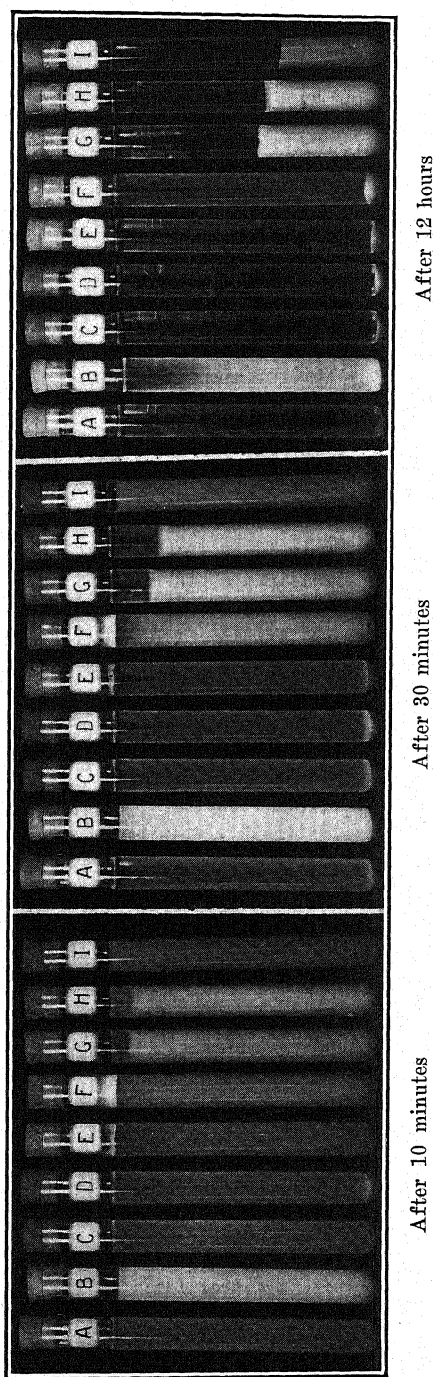


FIG. 2. Flocculation after indicated period. A. Check (no spreader); B. Tale; C. Bancroft; D. Kaolin; E. Flour; F. Fluxit; G. Wyojel; H. Bentonite, and I. Bordeaux.

TABLE 2.—*Copper residues. Comparisons of Wyojel and bentonite with basic copper sulphate*

Formula	Percentage residue sticking	
	Wyojel	Bentonite
2- .1-50	37.2	43.5
2- .2-50	43.5	47.0
2- 1-50	59.6	56.0
2- 2-50	94.96	95.5
2- 3-50	96.18	95.2
2- 4-50	96.7	96.3
2- 5-50	96.7	91.5
2- 6-50	96.2	73.1
2- 7-50	96.8	60.1
2- 8-50	96.4	64.8
2- 9-50	97.2	67.7
2- 10-50	98.1	59.6
Check—no spreader	33.8

the other clays. However, when greater quantities of bentonite were added, flocculation and jelling increased and adhesiveness decreased. On the other hand, Wyojel remained effective in all concentrations used.

Comparison of Bentonite and Wyojel with Coposil and Cuprocide

This test was set up in exactly the same way as the preceding ones. The formulae and results are given in table 3.

TABLE 3.—*Copper residue. Comparison of bentonite and Wyojel with Coposil and Cuprocide*

Spreader material	Residue			
	Coposil		Cuprocide	
	2-2-50	2-10-50	2-2-50	2-10-50
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Check	8.2	83.3
Bentonite	82.4	81.6	93.8	54.2
Wyojel	91.6	84.6	93.0	89.4

The results recorded in table 3 indicate that Cuprocide sticks very well alone. However, the copper content is high in this compound and for general use, particularly in dusts, diluents are necessary. Hence, it would seem that Wyojel would be far superior to the bentonite.

The 2-10-50 formula is comparable to the one advised by Wilson⁵ for cucurbit spraying or dusting; that is, 1 pound of insoluble copper, 5 pounds of diluent, and 1 pound of arsenical may be placed in either 50 gallons of water or dusted directly on the plants.

DISCUSSION

The selection of a spreader material for a particular spray is not an easy task. There is not enough fundamental information to enable spreaders to be classified as to their uses. When a fluffer or diluent is needed, such materials as kaolin, talc, Bancroft clay, gypsum, or hydrated lime are more frequently used. Most of them will serve as diluents but the other necessary qualities may be lacking. Lime is a good diluent but frequently reacts chemically with the fungicide or insecticide rendering it less efficient. Basic copper chloride and hydrated lime are less effective on potatoes for the control of early blight than the copper chloride alone.

There is also the large group of organic spreaders, many of which are extremely effective as stickers. However, they are also apt to be chemically active and change the effect of the spray, in some cases rendering it unsafe to foliage. Just because a spreader is effective in one material does not warrant its general use without preliminary trial.

When all spreaders are considered, the bentonites are perhaps the most widely used. They are practically inert chemically, adhere well to foliage, and are good adsorbers. Their jelling property causes a type of flocculation such as occurs in Bordeaux mixture, which gives excellent spreading and sticking. Their adsorbing property makes them useful when mixed with soluble or gas forms of fungicides or insecticides, such as zinc sulphate, pyrethrum, cryolites or fluorides, nicotine, etc.

The use of bentonite is further complicated by the variation within the group. There are many types of bentonites. Three types were studied in this work, one of which reacted much like kaolin or talc, had little jelling property in dilutions, and did not adhere well. The Wyoming bentonite (trade name Wyobond used here) gave excellent results. The Wyojel, a patented product, is simply Wyoming bentonite with a low percentage of magnesium oxide. This is added to increase jelling and adsorption.

DEPT. OF BOTANY AND PLANT PATHOLOGY,
OHIO AGRICULTURAL EXPERIMENT STATION,
WOOSTER, OHIO.

⁵ Wilson, J. D. New treatments for cucumbers. Ohio Agr. Expt. Sta. Bimo. Bul. 20(173): 68-75. 1935.

ON THE CLASSIFICATION OF PLANT VIRUSES

JORGEN M. BIRKELAND

(Accepted for publication July 10, 1935)

Johnson and Hoggan (6) have recently published a descriptive key for plant viruses that serves not only as a basis for classification but also calls attention to certain features of viruses most useful for diagnostic purposes. These features are:

1. Mode of transmission; 2. Natural or differential hosts; 3. Longevity *in vitro*; 4. Thermal death point; 5. Certain distinctive or specific symptoms.

Recent work on serologic tests and on induced plant immunity suggests that these reactions also may be of value in grouping viruses. The present chaotic state of nomenclature is due not so much to a lack of means of differentiation as to a lack of a suitable method for grouping viruses.

The serologic method, which has proved useful in identification of bacteria and in the discovery of their hidden relationships, has been applied to the study of a few plant viruses. It has been demonstrated that these agents are antigenic, that is, that they are capable of stimulating the production of precipitating, complement-fixing, and neutralizing antibodies when injected into rabbits. Furthermore, the antibodies are specific for a given virus (1), (2), (3), (5). Precipitin reactions and serum neutralization tests indicate (a) that the viruses studied fall into several distinct groups, and (b) that strains of the same virus are indistinguishable. Consequently, these tests are of particular value in grouping viruses. The relationship between strains of viruses is not always evident from a study of symptoms produced in the various hosts, since it has been shown that mutants or variants can be isolated that stimulate the production of symptoms quite different from those induced by the parent strain.

Mutation appears to be a common occurrence in certain plant viruses. By growing infected plants at high temperatures or by selecting portions from infected leaves showing different intensities of color, strains that "breed true" can be isolated. These strains, if encountered in nature, would undoubtedly be considered new viruses and would be named according to the symptoms produced on the hosts in which they are found. The pin-puncture and the leaf-excision methods used in isolating strains and the mechanical transfer of inoculum to healthy plants are more or less comparable to insect transmission. It seems probable, therefore, that insects in the course of their feeding serve as disseminating agents of variant viruses. This being true, we can expect a multiplicity of variants recognizable at first by different symptoms, but essentially the same by reason of

origin. To prevent confusion, the development of methods showing natural relationships is indicated.

The recent work of Thung (9), Salamon (8), Kunkel (7), and Caldwell (4) on immunity of plants from superinfection has shown that plants infected with certain viruses are immune from supposedly closely related strains but not from viruses that, according to certain criteria, are presumably unrelated. Caldwell has called attention to 4 types of response manifested by virus-infected plants when reinoculated with a second virus. They are:

“a. A virus may completely inhibit the development of another in the host tissues.

TABLE 1.—*A comparison of induced immunity reactions in plants with precipitin reactions of several plant viruses*

Virus used: (a) as serologic test anti- gen, and (b) to immunize plant	Virus used: (a) to produce anti- sera, and (b) to test plant for immunity	Reactions:	
		(a) Precipitin	(b) Plant
Tobacco mosaic	Aucuba (yellow)	+ ^a	Protection
Tobacco mosaic	Aucuba (green)	+	Protection
Tobacco mosaic, attenuated	Tobacco mosaic	+	Protection
Tobacco mosaic, attenuated	Aucuba (yellow)	+	Protection
Valleau's ring mosaic	Aucuba (yellow)	+ ^b	Partial protection
Tomato streak	Aucuba (yellow)	+ ^b	Partial protection
Spotted wilt	Aucuba (yellow)	no data	No protection
Tobacco ring spot	Aucuba (yellow)	—	No protection
Cucumber mosaic	Aucuba (yellow)	—	No protection
Healthy potato (Johnson)	Aucuba (yellow)	—	No protection but production of new symptom complex (streak)
Healthy potato (Johnson)	Tobacco mosaic	—	No protection but production of new symptom complex (streak)

^a Indicates a positive precipitin reaction. — Indicates no precipitin reaction.

^b Reciprocal precipitin tests were not run, so it is not possible to state whether these viruses are serologically identical. The results obtained indicate however that they do show a relationship.

- b. The second virus may multiply in the tissue without inducing typical symptoms.
- c. The two viruses may multiply and induce symptoms, each inducing the typical symptom of its specific disease.
- d. The effect of the second virus may be to cause a more severe disease than either could of itself have caused."

A good example of the last interaction (d) is "experimental" streak.

The parallelism between the serologic reactions of plant viruses and the reactions of induced or acquired immunity from those viruses in plants is striking. The following table is prepared from my own data on the precipitin tests and from papers by Caldwell (4) and Kunkel (7) on virus interactions. Viruses that induce a complete cross immunity in plants are serologically indistinguishable by the precipitin reaction; viruses that induce a partial cross immunity show serologic relationship; and viruses that do not induce cross immunity in a plant are serologically unrelated.

While these data are limited to a few viruses, their classification suggests the possibility of using such data in drawing conclusions relative to natural relationships of viruses.

DEPARTMENTS OF HYGIENE AND
BACTERIOLOGY AND BOTANY,
UNIVERSITY OF CHICAGO,
CHICAGO, ILLINOIS.

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INDEX TO THE RELATIVE SUSCEPTIBILITY OF ORCHARD APPLES TO CEDAR-APPLE RUST

IVAN H. CROWELL

(Accepted for publication June 20, 1935)

It is common observation by horticulturists and pathologists that the varieties of orchard apples differ in their susceptibility to the cedar-apple rust, and numerous varieties of apples have been reported as falling within certain groups or degrees of susceptibility. Two main methods have formerly been used to measure these degrees of susceptibility. Most prior investigators determined the relative susceptibility by a comparison of the number of lesions from visual observation. More recently Bliss¹ and Miller² gave accounts of statistical methods for determining relative susceptibility of orchard apples to cedar-apple rust. Their methods were based upon a count of the number of lesions on leaves of orchard apples.

It is common knowledge that the number of lesions varies in different years as well as in different orchards in the same year. It follows, therefore, that the results of such comparative and statistical methods of determining relative susceptibility also vary. Thus many varieties were reported by early investigators as belonging to 2 or more of the generally accepted grades or degrees of relative susceptibility, namely, immune, slightly, moderately, and very susceptible.³ Similarly, in the accounts of Bliss¹ and Miller² the statistical results varied for the same variety of apple. In fact, in Bliss's account the results of 3 different years varied greatly and were not in most cases consistent. Which values, therefore, are to be accepted as the measure of relative susceptibility?

In an account of this rust by Crowell,⁴ it was shown that the average number of aecia per lesion was directly related to the number of lesions on leaves of ornamental apples. Over a period of 4 years the number of aecia per lesion remained essentially constant, although in each of these years the

¹ Bliss, D. E. The pathogenicity and seasonal development of *Gymnosporangium* in Iowa. Iowa Agr. Expt. Sta. Res. Bul. 166: 340-392.

² Miller, P. R. A method of estimating the percentage of infection of apple leaves by rust with observations on the relative susceptibility of species and varieties of *Malus* and *Juniperus*. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rpt. 18: 159-163. (Mimeog.) 1934.

³ Crowell, I. H. The hosts, life history and control of the cedar-apple rust fungus *Gymnosporangium Juniperi-virginianae* Schw. Jour. Arnold Arboretum 15: 163-232. 1934.

⁴ Crowell, I. H. A compilation of reports on the relative susceptibility of orchard varieties of apples to the cedar-apple rust disease. Amer. Soc. Hort. Sci. Proc. 32: 1934. 261-272. 1935.

number per leaf⁵ varied widely. To test this principle on orchard apples, infected leaves of several varieties collected at various times from various States were examined. It was found that the average number of aecia per lesion for each variety was remarkably constant. The average number was taken directly as the measure of relative susceptibility. To illustrate: The Wealthy, one of the most susceptible varieties, averaged 22 aecia per lesion and its relative susceptibility was designated by this number. In obtaining data, lesions whose individuality were obscured by their proximity to the edge of the leaf, to larger veins, or other lesions, were not used.

This method of measuring relative susceptibility of apples to cedar-apple rust is free from the effects of seasonal and geographical variations in the amount of infection. It is based upon the physiological reaction between the host and the parasite. It was shown in the investigations on ornamental apples that the fungus developed most abundantly on the more susceptible hosts and less abundantly on the more resistant ones. This phenomenon has been repeatedly observed on orchard apples. Thus the relative susceptibility of any variety of apple may be determined from few lesions and is independent of the abundance of infections. The results of this method, however, are subject to minor variations due to variations in the health of the tree, the time of infection, and certain other environmental variables. A major variable may result from the presence of local or physiologic strains of the rust organism, but our present knowledge of strains of this rust goes little beyond the fact of their existence. It is to be expected that a small amount of variation in the number of aecia per lesion will be found. This variation may be minimized, however, by grouping, and the establishment of limits for each group. The generally accepted group classification and the limits now set for each of these groups for orchard apples are as follows:

Immune—No evident infection spots. *Slightly susceptible*—One to 5 aecia per lesion. *Moderately susceptible*—Six to 15 aecia per lesion. *Very susceptible*—Sixteen or more aecia per lesion.

These limits in the number of aecia per lesion are reduced from that originally proposed for the grouping of ornamental apples. The reduction is due to the circumstance that none of the orchard apples are so susceptible as the most susceptible ornamental apples.

To this series of groups another member may be added to embrace those hosts on which infection results solely in the development of spermogonia. Such infections have been vaguely interpreted as flecks, spots, specks, yellow areas, etc. These hosts are very resistant to infection. Scientifically they are susceptible, while, practically speaking, they may be considered as im-

⁵ A Correction: In my former article (1934) table V, p. 179, under heading *Number of cushions per leaf*, the 5.2 and 9.1 should be interchanged and under the heading *No. of aecia per leaf*, 375.2 should be replaced by 691.6, and 106.5 by 60.8.

mune, for, since they produce no aecia, they cannot reproduce the rust. This group may be known as the *spermogonial-host group* and should be placed between the immune and slightly susceptible groups.

This means of measuring relative susceptibility, based upon the physiological reaction between host and parasite, makes possible a classification of apples on a uniform basis, regardless of the number of lesions. Herbarium material as well as fresh specimens may be used. The results of infection in different years as well as from different localities may be directly compared and an accurate measure of relative susceptibility of orchard apples thus obtained.

PHYTOPATHOLOGICAL NOTE

Sclerospora graminicola on Millet in Minnesota.—During the summer of 1935 the writer made numerous observations and experiments to elucidate certain problems in connection with the development of *Sclerospora graminicola*. The results confirm some of the conclusions expressed by Hiura in his recent paper,¹ at that time unknown to the writer, and are summarized briefly in this note.

Sclerospora graminicola (Sacc.) Schroet. was abundant during 1935 on millet (*Setaria italica* (L.) Beauv.) and foxtail grass (*S. viridis* (L.) Beauv.) in the plots at University Farm, Saint Paul, Minnesota, and at the Coon Creek Experiment Station near Anoka, Minnesota. Primary and secondary symptoms of downy mildew, similar to those described by Weston,² were evident in both localities, but the primary were more common than the secondary. Yellow-green streaks running the entire length of the leaf of the dwarfed plant characterize the systemic primary infection, while the secondary infection consists of chlorotic local lesions (Fig. 1).

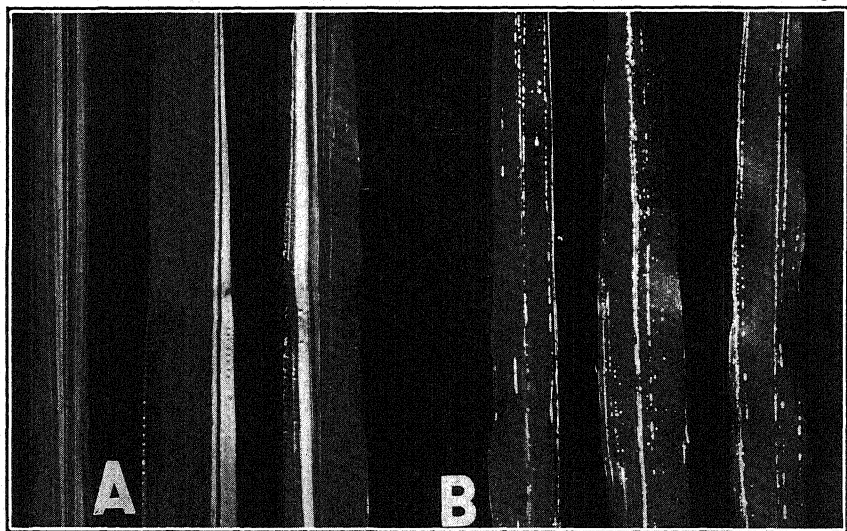


FIG. 1. A. Primary symptoms of *Sclerospora graminicola* on the fourth, fifth, and sixth leaves of an Italian millet plant. B. Secondary symptoms of *Sclerospora graminicola* on the fifth, sixth, and seventh leaves of an Italian millet plant. $\times \frac{1}{2}$.

¹ Hiura, M. Mycological and pathological studies on the downy mildew of Italian millet. Hokkaido Imp. Univ. Facult. Agr. Jour. 36: 121-283. 1935.

² Weston, W. H., Jr., and G. F. Weber. Downy mildew (*Sclerospora graminicola*) on Everglade millet in Florida. Jour. Agr. Research 36: 935-963. 1928.

Formation of conidiophores and conidia was observed in the millet plots at University Farm from July 15 to July 18, inclusive. Plants were examined at intervals throughout the day and night, and conidiophores and conidia were abundant on the lower leaf surfaces at all times and on the uppermost leaves of the plants, as well as on the lower. In order to determine the length of time required and the conditions necessary for spore formation, the old conidiophores were removed from the leaf blades with a cotton pad. A fresh crop of conidiophores grew through the stomata of the leaf within 4 to 6 hours, and conidia were formed 2 to 4 hours later (Fig. 2), the entire process requiring from 8 to 12 hours. A shorter time

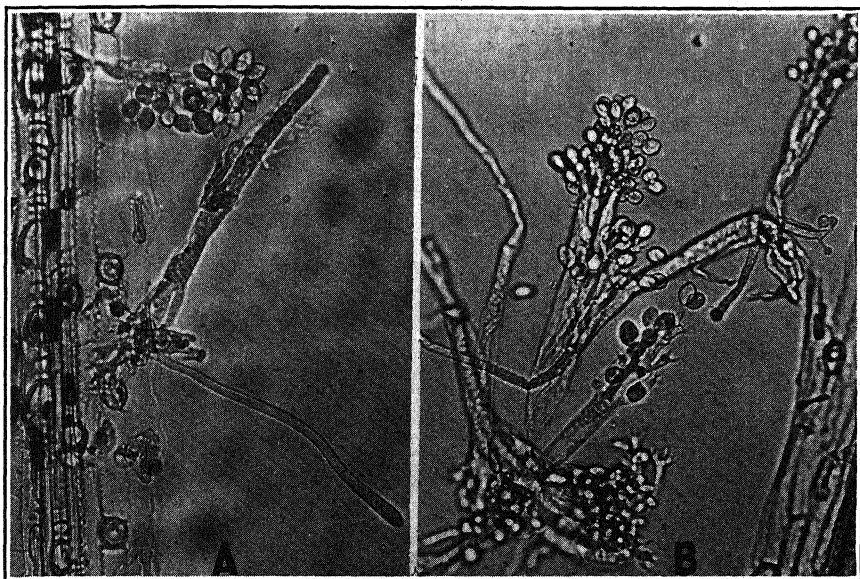


FIG. 2. A. Epidermis taken from the under surface of a diseased millet leaf at 10 a. m. showing young conidiophores growing out through a stoma, the remains of an old conidiophore, and a clump of conidia. B. Photomicrograph of conidiophores bearing conidia on short sterigmata. $\times 190$.

(6 to 9 hours) was required when the material was placed in a moist chamber in the laboratory. During the four days of field observations the writer found that conidia were formed during the day, as well as during the night,³

³ Weston reported that conidia were formed only during the night, but Melhus and his coworkers found them formed both day and night. See Weston, W. H., Jr., Nocturnal production of conidia by *Sclerospora graminicola*, Jour. Agr. Research [U. S.] 27: 771-784, 1924, and Melhus, I. E., F. H. Van Haltern, and D. E. Bliss, A study of *Sclerospora graminicola* (Sacc.) Schroet. on *Setaria viridis* (L.) Beauv. and *Zea mays* L., Iowa Agr. Expt. Sta. Research Bull. 111. 1928.

when temperature ranged between 17° and 34° C., and when relative humidity was between 75 and 100 per cent. Laboratory observations confirmed these field observations that agree in nearly all respects with the extensive report by Hiura.

One hundred conidiophores and conidia from *Setaria italica* were measured on July 17. The former were 182 μ to 251 μ long, with an average of 228 μ . The conidia averaged 21.4 $\mu \times 14.2 \mu$, while the 50 giant conidia measured were 43.0 $\mu \times 26.4 \mu$. The measurements of the fungus from *S. viridis* were slightly different, conidiophores averaging 184.4 μ in length, the ordinary conidia 19.2 $\mu \times 14.7 \mu$, and the giant conidia 40.0 $\mu \times 21.7 \mu$. Environmental factors are known to influence the size and shape of the conidia and conidiophores.

Mature conidia were washed from the leaf blades with sterile water. After 40 to 50 minutes in hanging-drop preparations or in Petri dishes at room temperature (23 to 30° C.) each conidium usually liberated about 4 zoospores. Some of them were killed and stained as in the method described by Cotner.⁴ The biciliate condition is typically found in the species. The biciliate zoospores remained active for approximately 30 minutes, then withdrew their cilia and encysted as globular resting bodies. Later, a germ tube was formed, and an appressorium usually occurred at its tip. The germ tubes died after 3 or 4 hours if there was no proper host for infection.—C. S. WANG, University Farm, St. Paul, Minnesota.

⁴ Cotner, F. B. The development of the zoospores in the Oömycetes at optimum temperatures and the cytology of their active stages. Amer. Jour. Bot. 17: 511-546. 1930.

PLANT QUARANTINE LEGISLATION—A REVIEW AND A REFORM¹

H. T. GÜSSOW²

INTRODUCTION

The serious economic losses resulting from the introduction of plant diseases or insect pests into the divers countries of the globe are so well known to plant pathologists and students of this subject, that no elaboration is required on this occasion. These often very costly and destructive invasions of a country's vegetal resources have given just cause for alarm. Let me briefly refer to the astoundingly large sums of money that have been spent on the Continent of America—notably by the United States—on two or three introduced diseases and pests since the days of their original introduction.

White Pine Blister Rust, *Cronartium ribicola* (*Peridermium strobi*), has involved expenditures in scouting and control of some \$14,000,000; the advent of the Gipsy moth, an expenditure of some \$41,000,000; while the European Elm disease, of comparatively recent introduction, has already caused an outlay of over \$1,000,000, which is likely to materially increase, if extermination is contemplated. Indeed, during the past decade or so, the Continent of North America has seen many alarming invasions—the San José scale, European corn borer, Oriental peach moth, alfalfa weevil, Japanese beetle, Mediterranean fruit fly, elm disease, blister rust, chestnut-bark disease, wart disease of potatoes and many others.

It is no wonder that, gradually, one country after another has instituted legislative measures governing the importation of vegetation of all kinds, both plants and plant products, likely to carry diseases or insect pests against which protection has become increasingly necessary.

It is of striking significance, however, that most of the diseases and insect pests mentioned, reached the Continent of North America since the adoption of such legislative measures, or the acceptance of guarantees offered by exporting countries by way of health certificates.

It is not my intention to review the efforts made by the various countries; I take it that these are well known to the group that I have pleasure in addressing; but, broadly speaking, these efforts either reflect an attitude of panic, or are mere first-aid measures to bring about what was thought to be a measure of safety and protection. While measures to control plant diseases, insects, or pests, bring profitable returns to those who practice them,

¹ Contribution No. 451 from the Division of Botany, Experimental Farms Branch, Dominion Department of Agriculture, Ottawa, Canada. Presidential address presented before The American Phytopathological Society, in session, 27th annual meeting, St. Louis, Mo., Dec. 31, 1935 to Jan. 3, 1936.

² Dominion Botanist, Department of Agriculture, Ottawa, Canada.

it is evident that they are carried out primarily for personal gain, and rarely to protect the resources of a neighbor or a community. Nor do I believe that such control measures are voluntarily practiced for patriotic reasons; that is, to protect the resources of the country. Measures taken with this object in view are usually the result of more or less forceful persuasion.

When it comes to the question of carrying out control measures solely for the purpose of protecting the resources of a foreign country, interest seems deplorably to be lacking, yet there exists hardly a problem in the realms of phytopathological endeavor the importance of which is so world-wide, and calls for such urgent, competent international effort, as the problem under discussion.

One of the first more concerted international attempts was made when representatives of the principal nations met in Rome as long ago as 1914, to discuss these problems. It was a laudable, but, as far as results were concerned, rather a futile attempt on the part of the International Institute of Agriculture, to bring the nations together. It was at that time realized that independent action by the nations seeking to protect their resources had brought about chaos, or, at least, an impasse; so the time was considered opportune for the consideration and adoption, if possible, of sounder and more logical principles or of the fundamentals of legislative measures that would prove acceptable as a basis for international action and coordination of effort.

I am impressed with the great divergence of opinion expressed at this Conference and with the scant results it has brought about. At any rate, the articles laid down at this Conference do not appear to have been ratified by the countries of the continent of America, notably Canada and the United States.

The solution of this problem—if a solution is practicable—throws a mutual responsibility upon every country, and as much as it is the aim of a country to protect its own resources, so it becomes the duty of competent plant pathological services to protect the resources of other countries, an attitude that has not always been very keenly observed.

A considerable amount of experience is necessary to deal with the perplexing problems constantly arising, and no doubt the Rome convention would have achieved considerably more had the wider experience of today been then available. But, even though the assembled nations at Rome in 1914 met with many difficulties, there exists no reason to postpone further attempts indefinitely. Valuable experience has been gained in the intervening years and the time seems opportune to plead for renewed international consideration of the problems in which all nations are vitally concerned.

The only feasible measures of protection are those based upon biological principles—a principle that is universally accepted by the technical experts—and that primarily aim at a thoroughly satisfactory protection of any and every country's agricultural, horticultural, and vegetal resources, without subjecting any one nation to further risks or expenditures, or leading them into difficulties generally in regard to trade and commerce. Such results can be achieved only when all countries alike are prepared, and—above all—properly organized, to assume their share of responsibility, not only in seeking to protect their own resources, but the resources of other countries, as well.

I. PLANT QUARANTINE LEGISLATION: A REVIEW

Aside from a number of minor or specific ordinances, there are at the present day three outstanding measures in operation, which, it was hoped, would bring about satisfactory results, *viz.*: the total embargo, health certificates and restrictive measures. The first is an attitude of watchful waiting; the second an attempt to throw the sole responsibility upon the exporting nation, without due regard to the need for protection of importing nations; and the last a more or less happy compromise of the other two.

1. *Embargoes Generally.* It is only in rare instances that a country resorts to the prohibition of importations of the commodities in which we are interested unless it has had some definite experience with a particular disease or insect pest. In most cases an embargo is the result of a more or less serious experience that a country has had and against the repetition of which it desires to protect itself. In rarer instances, a group of countries, suitably located geographically, will agree among themselves to place an embargo against other countries and their products that may involve them in serious losses. I shall return later to the question of groups of countries.

Usually, the detection of a disease or pest suspected of foreign origin—and which pests or diseases are not nowadays considered of foreign origin?—will lead to an embargo. I should like, at this point, to refer to the regrettable eagerness shown by some investigators to brand a newly discovered disease or insect pest as of foreign origin. I maintain that this attitude is unworthy and illogical and one that cannot too strongly be deprecated. In a country that is comparatively virgin as regards taxonomic studies, a disease newly reported may not be one of recent introduction, but may have existed, unknown, in the country for many years. Taxonomic studies have been carried on, especially in Europe, for a century or so and that the “new” disease has been described there is of little significance. To think otherwise verges perilously on the absurd: “*Post hoc ergo propter hoc*,” and it is still more to be regretted that this attitude, once adopted, is not readily abandoned, even after the discovery that the new “foreign” disease had been in the country for years. To anticipate my critics I wish to state

that I am quite aware that certain diseases, insects, and pests have been introduced from one country into another, and may justly be regarded as of foreign origin.

To return to the question of embargoes. There are a number of completely justifiable embargoes in force today and careful consideration may lead to others. Why, for instance, should Canada and the United States have imported seedlings of native trees such as *Pinus strobus*, *Pseudotsuga douglasii*, of *Larix laricina* and other genera—by means of which some new diseases or pests have actually been introduced? In the case of blister rust of five-leaf pines it seems extraordinary, that notwithstanding the abundant records at our disposal of the disastrous effects of this rust on white pine introduced into Europe from this Continent, importation of white pine nursery stock was carried on until—alas—this destructive disease gained a firm foothold both in the United States and Canada, leaving little hope for its extermination. In many cases the embargo has come too late. Furthermore, there is this very important question: Does an embargo effectively protect the resources of a country? Is it practicable, with modern facilities of transport, to guard effectively against pests or diseases by this means or to supervise adequately all methods of importation? As a matter of fact, even though embargoes have been instituted and maintained by all practical means, convincing and abundant evidence exists, that certain insects, pests and diseases have become established in the country maintaining the embargo. We have recent evidence that diseases and pests have been brought into a country by vegetation not intended for propagation. Plant products are now used industrially or for manufacturing purposes to a much greater extent than before and to make an embargo completely effective by closing all channels through which desirable or destructive diseases or pests may be introduced seems as economically difficult as it is practically impossible. No one could possibly have anticipated that manufacturers of furniture veneers would, innocently enough, cause the introduction of the European elm disease into North America by means of “burls” (knots) or elm logs used for the manufacture of veneer.

Europe has had for many years an embargo against potatoes from countries where the Colorado beetle existed, but the Colorado beetle has finally made its entry into European territory. The entry, apparently, was not made by means of potato tubers—but Europe persists in maintaining the embargo.

There exists a further aspect to embargoes. Only in rare cases may topographical location assist, as in the case of islands or areas protected by mountain barriers, etc., and though it is true that many diseases and pests have been introduced into other countries by the commodities at present subject to certification, the more recent epidemiological studies have thrown an en-

tirely different light upon the situation, by indicating vitally important factors over which one cannot hope to exert any material control.

The researches at our Winnipeg Laboratory in connection with stem rust of wheat have conclusively proved that the spores of this rust may be carried in the air for tremendous distances and at surprising altitudes. In certain years, stem rust no doubt winters over in the southern States of America, and spores are carried northward until they eventually reach our own grain-growing areas in Canada. You are no doubt familiar with the technique followed in which aeroplanes and ground spore traps played an important rôle, detailed accounts of which have been published.

While this phase of research is perhaps second to none in regard to accuracy and deduction, there exists little doubt that the time has come for further intensive epidemiological studies in which all nations should definitely cooperate. In view of the results obtained, there is little doubt that other diseases, insects and pests, have been dispersed in a similar manner—let me just instance the sudden and wide-spread outbreak of oak mildew in Europe some years ago. If such occurrences are more frequent than our present knowledge would indicate, embargoes may certainly be limited in their effectiveness. Thus, on a continent like Europe, the many international boundary lines do not constitute the slightest barrier, and embargoes between countries of one continent cannot serve the object for which they have been devised. These investigations clearly indicate the importance of a change in attitude and the substitution of entirely different methods from those relied upon at present. But with this I shall deal later.

There is a further aspect of embargoes, less biological in nature it is true, but one that has elicited frequent criticisms, *viz.*, that embargoes may be placed, and no doubt actually have been placed in many instances, to exclude commodities from a market in order to gain a trade advantage.

This also is emphasized by Dean Hutchison of the College of Agriculture of the University of California, who remarks that "such procedure is not an honest application of the principles of plant quarantine," and I think we may leave it at that. Should political or economic expediencies require an effect of this kind then the matter could be more honestly dealt with by tariff boards or similar organizations. There is this suspicion, though, in the minds of many—a suspicion almost impossible to confirm, but which might be allayed by an international council of arbitration to assist in deciding whether any such embargo may be warranted for strictly biological reasons.

Several countries have taken the lead recently in removing embargoes when it was shown that biological reasons no longer existed for them. In this connection it may be stated that not so many years ago nursery men and related interests strenuously opposed plant quarantines, claiming that they interfered seriously with their business transactions. The measures, how-

ever, proved to be a valuable means of protection for that industry, and subsequent proposals to modify them to bring them more in harmony with biological principles were opposed just as strenuously by the same interests.

It will be seen that the question of embargoes is exceedingly far-reaching and one that requires unbiased and serious consideration in the near future, since their enforcement does not always achieve the expected results.

2. *Health Certificates.* Much has been said and may be said on the subject and value of health certificates, particularly of the type in common use today. A careful consideration of all the relevant facts and experiences suggests that the guarantee attached to the certificates by the services issuing them has but little value. The laxity with which some of them are issued, or the indifference in regard to their true meaning, would indicate that facilitation of export seems the main issue involved. For it has long been the somewhat apologetic practice to certify that a consignment is "reasonably or apparently free from" or has "been examined by an authorized inspector who failed to find any evidence of" any (specified or not specified) diseases, insects, or pests. These reservations clearly indicate the limitation of health certificates and merely endorse the fairly universal opinion that they are only instruments of relative guaranty; exporting countries being more convinced of their value than importing countries.

It is admitted by any one who has had experience in the examination of vegetation, plants, or plant products, that it is not possible to issue a health certificate with any degree of accuracy, indicating that a consignment of nursery stock, bulbs, scions, cuttings, etc., is free from even specified diseases or pests, apart from any that are not specified. Certification usually takes place immediately prior to export, and since export of these commodities is crowded into comparatively short periods of time, the rush militates against thorough examination. Moreover, most of the diseases and many pests are of microscopic nature; many of them are dormant in a dormant vegetation, or may require definite periods of incubation before showing signs of their presence (as for instance blister rust of white pine), conditions that render certification extremely difficult and decidedly uncertain.

Furthermore, the most careful inspection immediately prior to export, *i.e.*, after nursery stock has been removed from where it grew, or after bulbs, etc., have been harvested, is scarcely more reliable than one made at the port of entry. As a matter of fact, all vegetation, plants, or plant products exported from one country to another, should be free from any disease or pest. No one would deliberately introduce nursery stock or similar material covered with oyster shell bark louse or *Nectria* fructifications, or their roots affected by nematode galls, even though these troubles are almost universally prevalent in countries of similar environmental conditions, especially when vegetation free from these common pests may be available. The inadequacy

of certification before export becomes still more obvious when the prevalence of seed-borne diseases is considered. Certification has never been applied to "agricultural seeds," mainly because of the opinion that such application would seriously interfere with trade, biological necessity being completely disregarded. Without wishing to cause undue alarm, I may state here that recent investigations conducted in our own laboratories and elsewhere have clearly revealed that even "elite" seeds may carry destructive diseases. This disturbing discovery may cause an entire change in attitude in reference to seed production and seed certification. It is the plain duty of plant pathologists in every country to combat diseases of all kinds, and to dismiss an important factor in the dispersal of plant diseases as one about which nothing can be done is an admission of failure. Indeed, during more recent years, this question has received considerably more attention from countries whose resources have been gravely endangered by diseases, and, to a lesser degree perhaps, by insect pests, introduced through seed; such diseases, for example, as blue mould of tobacco, cotton wilt, bacterial wilts or diseases, loose smuts, flag smut, and bean or pea spots. The present system of certification, however, cannot very well be applied for seed-borne diseases. The problem is a difficult one but of such economic significance that it demands precise and immediate study.

Another problem that also renders the present method of health certification obsolete, has arisen during recent years, and is of equal, if not greater, economic importance. I have reference to virus diseases affecting plants. The importance of considering immediate action in regard to these diseases was recently brought to our attention in the case of a serious virus diseases detected in *Iris tingitana* imported from Europe and in *Lilium longiflorum* from the Far East. Other virus diseases in dormant bulbs also are known. Narcissus species and varieties, notably the variety Lucifer, apparently is never free from a mosaic. "Breaking" in tulips, constitutes a menace to growers of self colors, and many more instances of more recent manifestation might be quoted. This virus problem hardly requires discussion, since it is obvious that no present health certificate is of the least value in this connection and that entirely different practices will have to be substituted.

3. *Conditional and Restrictive Measures.* These measures are largely a compromise between total embargoes and usual health certification, and may be said to meet many cases most satisfactorily. They, at least, offer a guaranty by rightly placing very definite responsibilities upon the plant pathological services of a country; a guaranty, for instance, that certain diseases or pests are not present in a country at all, or not in any defined district or territory thereof, or have not been present for a stated number of years or within a certain radius whence they originate. Yet these measures are equally subject to abuse and the provisions may be so severe or impractical

that they amount to an embargo. Personally, I consider the honest application of such restrictions as a sound policy—one, at least, of “the open door” and one likely to stimulate more competent and efficient inspection services in all countries.

Indeed, it would be a wise policy to devise means to stimulate the phytopathological services of any country to greater and more efficient effort that will result in an advancement of trade rather than interference with it—an action that would immediately commend itself to any government and thus cause a more ready financial support of such technical services. Just in passing it may be said that any inspectional service that can show evidence of promoting any healthy export would not find it difficult to secure support from their administrations, which today are becoming more and more reluctant to act, simply because of indications that the present measures in force are inadequate, interfere with legitimate trading, and eventually cause retaliations of all kinds.

Any critical consideration of the international phase of plant diseases, insects, and pests must eventually lead to the realization and admission that our knowledge of control measures is far from complete; and also, perhaps, that producers do not always carry out efficiently those measures that are known to be adequate. It goes without saying that if it were possible to control, or better still to eradicate, the diseases, insects, and pests that affect commodities of international trade, all regulations and restrictions would be much simplified and trade materially enhanced. As a plant pathologist, I frankly admit that the greatest of our problems, namely, the determination of effective control of plant diseases, has on more than one occasion been sacrificed to the more academic phases of research in plant sciences, in spite of the fact that our services exist primarily for the purpose of benefiting and promoting our industries.

Finally, critics of the suggestions made to further much needed reforms, should bear in mind that the difficulties that may be met in implementing the proposals may be largely due to the inadequacy of the control measures recommended, and thus emphasize the necessity for further research. They certainly do not justify the arbitrary dismissal of the suggestions on the ground that they are impracticable. Measures that are feasible as far as plant diseases are concerned should be equally applicable to insects, but the fact that the methods of the phytopathologist differ from those of the entomologist is surely no reason whatever for delaying action along a front that leads itself more readily to attack.

II. PLANT QUARANTINE LEGISLATION: A REFORM

The foregoing review has elicited a number of points that require future and serious consideration. Of the suggestions for reform that I take the


privilege to recommend, I would place foremost the organization in all countries of adequate and reliable plant disease surveys.

Plant-Disease Surveys. One of the responsibilities that countries mutually bear is the organization and maintenance of adequate plant-disease (insect pest) surveys, so that these services, if called upon, will be able to provide reliable and exact knowledge of any disease or pest and of its geographical distribution, and thus delimit areas that have remained uninvaded or have been effectively freed from it. The plant pathological service of each country should prepare a periodical report of such surveys for distribution to all countries that may be interested in any commodity to be exported. Unless such services are performed honestly and with sincerity as a measure of effective international cooperation, they will be of little value. It would seem urgent that all countries that have not already done so, should establish these services as one of the fundamental requirements for international intercourse in these commodities.

It is of interest to refer to a resolution passed by the Pan Pacific Congress held in Vancouver, British Columbia, in 1934 endorsing "the desirability of cooperation in the compilation and sharing of data relating to plant disease survey work." Obviously, opinions may differ as to what constitutes an adequate plant-disease survey, but this should cause little difficulty as long as one bears in mind that the published records should provide useful information to all countries concerning the prevalence and economic importance of diseases, insects, or pests. It would seem desirable to deal with this question from the international points of view and to arrive at last at an agreement as to what are the purpose and aim of such surveys and their records.

Material other than Living Plants. In view of the recent introduction of the European elm disease by means of elm burls imported for the manufacture of veneer, and of corn-borer larvae in broom corn from Europe and other similar experiences, it seems to be important for all countries to supervise more carefully the export of all kinds of materials of this type, including packing materials, boxes, barrels, hoops, etc., so that no product of the vegetable kingdom may become the source of establishing diseases or insect pests elsewhere. Here again discussion by an international committee of experts is most urgent.

Urgent Necessity for the Supervision of Exports. Summarizing the experiences of the past and looking towards greater cooperation in the future, it becomes obvious that all countries should make it their foremost concern and duty to prevent the export of any kind of materials, vegetation, plants, or plant products, including seeds and dormant bulbs, rhizomes, corms, tubers, etc., likely to be infected by diseases or insect pests that might prove of serious menace in any other country—or specific groups of countries.



Such action has become increasingly important in view of the nature of seed-borne and virus diseases, as I have pointed out previously.

I cannot see any other solution to this problem than the organization of competent inspection services whose primary duty is to inspect the plants, bulbs, etc., during the growing season at the actual place of growth and culture, and to issue certificates entirely based upon their findings. In order to give such services proper legal standing, it is paramount that all countries should make the necessary provisions under their respective Destructive Insect and Pest Acts, to enable them to restrict the export of all such commodities to plants found sound during the growing season, and to definitely interdict the export of diseased vegetation. The Dominion of Canada probably has taken the lead in this connection by an amendment to its Destructive Insect and Pest Act enabling the administration to make such regulations as are deemed expedient to prevent "the shipment beyond her borders" of any insect, pest or disease destructive to vegetation, and it is hoped that other countries will see the wisdom of such provision for the benefit of all countries and international trade. I think we shall all readily agree that inspection at shipping time alone is useless as a measure against virus and seed-borne diseases, and that neither the usual export certificate nor the port-of-entry inspection is of much value. In consequence, it is desirable that no country shall permit its public carriers, mail, express, or freight services (including auto and aeroplane and all other means of transport) to accept for export beyond her borders any commodity (to be defined) unless specific authorization has been given by responsible authority.

In order to facilitate supervision of export under these conditions, it is suggested that all bona fide export firms, which preferably should be the original producers, shall make application to the plant pathological service for registration. Following such application the plant pathological and entomological services shall become responsible for the thorough, periodical, and competent inspection of the commodities produced, and, on satisfactory evidence of health, shall grant to any such qualifying concern a license to export. It is impossible here to go into further details of such services and of their duties, and the conditions under which such export licenses be granted.

There is one point though to be emphasized, *viz.*: should at any time an exporter be refused a license to export, it will become his immediate duty, and be very much to his advantage, to put into practice the most up-to-date and effective means of disease or pest control, advice on which it shall be the duty of the plant pathological and/or entomological services to impart. It is obvious that such practices would very materially improve the general health condition of such commodities throughout an entire country.

As a matter of fact, I am in a position to indicate that the measure proposed has been put to a severe test with certain commodities, and it has been proved that its value greatly exceeds that of any other measure in force at present. In Canada, such procedure has been put into practice and has been maintained for some years with evident success. I refer to the seed-potato certification service, initiated primarily as a control of virus diseases, but also cognizant of any kind of potato disease. In 1915, when the service first started, certified potato fields averaged 12 per cent combined diseases; after seven years field inspection work, the average total of the three principal diseases (leaf roll, mosaic and black leg) found in all fields, had been reduced to about 6 per cent. In later years the averages were:

1923	Leaf roll .44	per cent	Mosaic 2.8	per cent
1933	Leaf roll .17	"	Mosaic .98	"
1934	Leaf roll .16	"	Mosaic 1.02	"

The many years of success of this type of service indicates its practicability and, incidentally, shows that virus diseases may be kept reasonably from increasing. Indeed, many fields are found every year that are totally free from all kinds of diseases.

Similar success has been attained in the certification of raspberries and, in other countries, of strawberries and other crops. We now contemplate the inauguration of similar services for the certification of bulbs, etc. While, no doubt, difficulties will be encountered in the early stages of such services, these should not constitute obstacles, but rather stimulate intellectual approach. The problem not only deserves but urgently calls for serious cooperative effort.

Tolerance and Standards. After the work of field and nursery inspection has been completed, the products or commodities originating from establishments passed for export, shall be further inspected and certified in regard to diseases or pests that are apparent only after digging operations (root galls, nematode infestation, scab, rhizoctonia, etc.). In this connection it may be mentioned that certain countries have (already) set more or less reasonable domestic standards and may naturally expect imported products to at least conform to these.

While it is highly desirable from every point of view to aim at the complete freedom of vegetation, plants, or plant products, etc., from diseases and insect pests, it is hardly reasonable to expect such perfection at this stage of our knowledge, and of our respective organizations. The attempt to vouch for what cannot practically be accomplished has discredited many a certification service, and we must be content to make it our first aim to protect the resources of other countries as honestly as our own. This may be commenced by devising limits of tolerance, especially for diseases and insect pests widely prevalent throughout geographical groups of countries.

Thus, countries within the temperate zone, where potato scab, rhizoctonia, oyster shell bark louse and similar troubles are universally prevalent, should mutually determine the tolerance or maximum extent of any such disease or pest that will be permitted on imports. Let me give, for instance, a tolerance allowed for potato scab:

Slight infection, where the coverage is from 1 to 5 per cent of the surface, allowed on not more than 10 per cent of the potatoes.

Moderate infection, from 5 to 10 per cent surface coverage, allowed on not more than 5 per cent of the potatoes.

Severe infection, with more than 10 per cent surface coverage, no allowance.

This standard has served our purposes quite satisfactorily in the case of the production of certified seed potatoes and is liable to be adopted throughout the continent of America as a standard for scab tolerance. It may meet the needs of other countries, too.

Similar standards of tolerance are required in regard to the more universally present troubles, and geographical situation may materially simplify such determination. Some countries have no such standards, while others have regulations that practically debar everything from being imported, so that a clear understanding seems urgently required. One experience comes to my mind where a certain country rejected a high grade of potatoes on the ground that they did not "conform to their standards," but showed 2 per cent scab, 1 per cent silver scurf (*Spondylocadium*), and 1 per cent *Fusarium* dry rot. On inquiry we ascertained that the port of entry inspection service based its decisions on the conditions, amongst others, of freedom from bacterial soft rots, "filtrable viruses" and "worm cuts." I prefer to make this statement without further comment, but the practice of this and other countries seems to confirm the opinion that these difficulties might be overcome by agreeing upon tolerance limits that are more acceptable internationally. I am quite aware of the problems that may arise in setting such standards for certain diseases and insect pests, but with the usual spirit of understanding among nations, these difficulties should not prove unsurmountable.

Port-of-entry Inspection. Just as the suggested system of field inspection and certification is bound to relieve the "pre-shipment" inspectors of a country of much responsibility, so will it relieve the port-of-entry inspection services of the sole responsibility of passing materials offered for import.

If, furthermore, all export consignments were officially tagged to identify the country of origin and the certification authorities, and were found duly in order, the port-of-entry inspector would have to examine only a certain percentage of imported material. This, indeed, is the practice today, but

it has not the measure of protection behind it that is now suggested. In the case of bulbs, rhizomes, etc., duly furnished with field inspection certificates in regard to virus diseases, the inspectors should note the destination, and request information on the quality shown by such commodities on growing or forcing—to be confirmed where necessary by an official of the service. Unless such procedure is followed, virus diseases will continue to invade new territories, and since they are undetectable in dormant vegetation, the port-of-entry inspection service will, most unjustly, be exposed to complaints and claims for having passed inferior material. It should, however, be part of the routine of port-of-entry inspection services to refer all complaints of this nature to the inspection services of the country of origin, submitting samples where possible, and requesting immediate adjustment through the producer or other liable concern. A prompt official report on the action taken should be submitted in each case.

Insurance Against Interceptions. This proposal does not directly concern plant-disease legislation but is closely related, and is submitted for consideration by the more or less purely commercial interests. Experience has shown that notwithstanding a health certificate issued by the country of export, and a “port of entry” inspection, certain classes of vegetation are often released that do not conform to the expectations of the importer. They may be free from diseases or insect pests, but may have suffered in storage prior to shipment, have become injured during transit by heating or salt water, or may be infected by viruses or other troubles not recognizable by even the most competent port-of-entry inspector. The effect is that the consignment proves of little value, and is not acceptable to the importer. Such occurrences are not uncommon. They invariably give rise to complaints, and lengthy correspondence involving expert advice, and in some cases have led to arbitrary decisions, unsatisfactory to both the exporter and importer. It is suggested that the matter be taken up with insurance companies, who may be prepared to insure exporters against such risks, covering partial or whole destruction or interceptions of this type.

Quarantine and Detention Services. Realizing that port-of-entry inspections have definite limitations, that many troubles cannot be determined in dormant matter, and that microscopical examination is impracticable because of the delay, and of the large force of inspectors that would be required to deal with the rush at seasons of import, the suggestion has been made that quarantine stations be established or, at least, provision made for more adequate detention services through which questionable introductions may be safely held for observation. This system has been practiced with success in health of animal services and it seems only logical to consider such arrangement in health-of-plants services. Indeed, such practices have been followed in some parts of the British Empire and elsewhere, notably with

citrus fruit, bananas, and sugar cane; but the value of such detention stations would be limited to the quantities that could be safely dealt with, and to very specialized plants or products. At any rate, it would seem worth while to consider carefully the feasibility of this proposal and to define the types of importation to which it would apply.

The great difficulty will always be to know how to safeguard against escapes or how to prevent the introduction of plant pests or diseases brought in by other means than living plants or products. Technically, difficulties are bound to arise. It is well known that temperature and environmental conditions generally, influence the development of microorganisms, and, unless adequate variation is provided, may give rise to the development of less important organisms, while inhibiting the growth of more serious ones. Moreover, in the case of valuable tropical orchids, competent care is essential to maintain growth, and the failure or death of any such material may involve an administration in rather costly compensation or litigation, a predicament that should be avoided at all costs. Personally, I lack experience in the operation of this system, but I fear that such undertaking may considerably add to the cost of maintenance of plant pathological services.

Agreements among Groups of Countries of Similar Interests and Geographical Location. I have already emphasized the urgency of a mutual agreement between countries on regulations governing import and export of commodities under consideration. The present impasse has seriously affected international trade, and even good will, since it has led to threats, or the actual levy, of protective tariffs on commodities other than plants and plant products. An important step to this end would be taken by the alliance of countries of identical or similar interests and of suitable geographical locations for the adoption of measures protective of the resources and interests of the whole group. Thus, the countries of North America, namely the United States of America, Canada, and Mexico, have many common interests and would mutually benefit from united action. These countries have actually had some sort of group agreement in operation for a number of years, especially relative to diseases, insects, and pests from European and Asiatic countries. Another group might be formed by the countries of Latin America, including, probably, Central America; and the countries of Europe might find it of advantage to unite to devise cooperative means of protecting its entire area from invasion of specific diseases from other continents. Some of the British overseas possessions have already such an agreement in force and have been joined by possessions, suitably located, of other countries.

I believe that such group systems would promote international understanding and good will, and are deserving of the most intensive study.

International Cooperation in Scientific Research. I have repeatedly referred to certain reforms that require a great deal of careful thought and

research before they can be put into operation. There cannot be any doubt that a very cordial relation has existed among the plant pathologists of the world, but it does seem that scientific efforts should be more closely coordinated, particularly in its bearing upon the perplexing problems under discussion. In Bulletin 553 of the University of California, a similar thought is expressed: "Intensified efforts must be devoted to the development of methods for the treatment of agricultural commodities to free them from pests and diseases to the end that they may move freely in the channels of commerce, in this way removing so far as possible the necessity for embargoes. In this direction lies one of the most important possibilities of reducing plant quarantines; carriers thus forestalling serious interferences with the marketing of crops and the same time preventing so far as reasonably possible the spread of dangerous pests and diseases."

It may be said that international effort has been materially assisted by the dissemination of information through periodicals and the more or less critical abstracting journals; but its success depends largely upon the close contact of the research workers themselves. To promote this association and at the same time to obtain the greatest amount of benefit from the work accomplished, every facility should be given to the international exchange of workers specifically charged with duties pertaining to plant quarantines. The question of accepting, with safety, the verdict of an official is decided largely upon individual reputation that he has established, and to know, personally, the responsible officers in charge of these services in the various countries would be of distinct advantage and would aid in cooperation.

This exchange of workers has been the practice in related services in the British Empire and the United States, and any administration would be well advised to send its technical experts from time to time to other countries to become familiar with the way specific disease problems or pest control are handled. Considerable saving may eventually be effected by this policy, as much expenditure in speculative fighting of foreign pests or diseases may be avoided. Indeed, it would be worth while to consider the advisability of attaching competent officers to the consular services in the principal countries from which imports are drawn, so that specific information on foreign diseases may be readily available from experts familiar with such problems.

Remarkable progress has been made during the past decade or so in relation to breeding plants resistant to certain diseases and in regard to biological control of insect pests. While in this latter aspect, international cooperation has been developed to an appreciable extent, it is doubtful whether similar cooperation exists in the interchange of vegetation resistant to diseases—or, incidentally, to drouth and frost. Furthermore, since the days of 1914, there has been much work done on biological or physiological strains or races. In Canada and the United States particularly, much progress has

been made in the study of physiological specialization of stem rust of wheat; and at present several varieties of wheat that are quite immune from this most serious of all rusts are being propagated for distribution. We know, however, of the existence of strains of rust in other lands, the introduction of which would seriously compromise our efforts to produce a resistant variety of wheat for our conditions. So far we have, with one exception, only comparatively harmless strains of crown rust of oats, but other countries have forms, which, if introduced into Canada, might cause serious losses. As far as we know we have no physiological specialization in *Phytophthora*, but in Germany such races have been discovered.

This introduces an additional phase for international cooperation, *viz.*: Research on the behavior of plants, immune from the physiological strains of one country, to those of another, and the application of the knowledge gained to the problems involved. Any cooperation dealing with the testing of native crop plants in a foreign environment to determine their reaction to diseases not yet known in the country of origin is much to be advocated. To some limited extent such cooperation has been actually put into practice between the United States and the Dominion of Canada in an attempt to learn something of the reaction of species of *Grossularia* and *Ribes* native in California and along the Pacific Coast of the United States to *Cronartium ribicola*, which has been prevalent in the limited five-leaf pine resources of the Canadian Province of British Columbia.

The question of epidemiological studies has been referred to previously, and it would seem to me that this problem, too, is one for international cooperation. Attempts to determine the fungus spore content of the air between ports of Canada and Great Britain have been made, many times on steamships, and also on the British dirigible R100 during its first flight over the Atlantic, for there are so many striking instances of the sudden arrival of diseases or pests in often remote sections of a continent, that the distance flight of fungus spores may well account for them.

The problems of virus and seed-borne diseases have not received the attention they demand, although some work has been done on them in certain countries. Even though these problems are complicated, they should not be neglected, and if the reforms I have suggested prove to be acceptable, solutions should not be impracticable. Both problems should encourage cooperation among the plant pathologists of the world.

International Advisory Council on Plant Quarantines, Suggested. Countries that export extensively have felt the economic consequences of quarantines very keenly; far more so, of course, than countries with restricted exports. It is surprising to find countries maintaining many restrictions or embargoes that are biologically quite unsound or, at least,

unreasonable. This attitude has caused many a country real concern, and in some instances has led to retaliatory measures that have not improved the relations between countries. The administrations of many countries feel that plant quarantines of the present day are more or less a serious nuisance, but few of them have shown a readiness to support the maintenance of a responsible international council to review from time to time the whole progress made in this connection and to suggest improvements to facilitate international trade in these commodities. Some such attempts have been made from time to time, but I am not aware of any active interest that has been taken in the attempts by the governments of many countries. It is, therefore, suggested that such an advisory council should be subsidiary to some outstanding organization of international repute, and I certainly think that the Council of the League of Nations should be officially requested to promote an organization of this type with well-defined duties as indicated by the many problems and difficulties confronting us. The League of Nations has actually, as occasion warranted, appointed committees of experts to consider similar problems, as, for example, the "International traffic in animals and animal products." It is pointed out in a report of this committee, that, if one nation controls or eradicates any particular disease, a benefit is thereby conferred on all nations, while its presence inhibits or restricts international traffic. This, exactly, is the well-established principle upon which the suggested reforms are based. Since the first attempt was made in 1914 for international action in reference to diseases and pests affecting plants and plant products, a sufficient period of years has elapsed and considerable experience has been gained, to enable the problem to be examined in a true perspective. "In the League of Nations and the expert committees which the League may appoint, there exists the required potent agency to bring about that international cooperation which is such a dominant factor in the success or failure of the campaign against disease." It is to be hoped that international consideration may be given to our problems in the near future, for the continuation of our present practices will make future contacts among nations still more difficult.

In conclusion, it may be of interest to record that action was taken both by the plenary sessions assembled on the occasion of the Sixth International Botanical Congress, Amsterdam, 1935, and the American Association for the Advancement of Science, St. Louis, 1936, in forwarding the following resolution, which I had the honor of proposing on both occasions:

The members of Section: Phytopathology express themselves of the opinion: "that an effective and unceasing campaign against destructive plant diseases, insects and pests can only be successfully prosecuted by international action and mutual cooperation . . .

“that close and frequent international discussion of the problems of plant quarantine should take place to bring about improvement of the health conditions of plants and plant products offered for export,

“that they unanimously recognize that such action will greatly facilitate international trade in the commodities concerned; and

“that, finally, the matter shall be brought officially to the attention of the League of Nations with the recommendation that the matter receive the careful consideration of that organization, with a view to facilitating and expediting the purposes and aims of this resolution.”

REPORT OF THE TWENTY-SEVENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE ST. LOUIS MEETING

The American Phytopathological Society held its 27th annual meeting at St. Louis, Mo., from December 31, 1935, to January 3, 1936, inclusive. Headquarters were at the Statler Hotel. Sessions were held at Washington University. The meeting was well attended. About 160 members and 50 visitors registered. For the first time, by decision of the Council, the meetings were extended over 4 days instead of 3 in order to relieve program congestion. Speakers also were held to time, a requirement that met with unanimous approval, since it afforded more opportunity for discussion.

The program was of outstanding interest and value. There were 132 papers and addresses presented in 14 sessions, including joint sessions held with Section G of the American Association for the Advancement of Science, the Mycological Society of America, the Genetics Society of America, and the Potato Association of America. These joint sessions were much appreciated for the stimulating viewpoints and broad perspective contributed by the representatives of different scientific and applied fields. The programs of the joint symposium type proved so worthwhile that their continuance appears highly desirable.

The address of the retiring President, H. T. Güssow, entitled "Plant Quarantine Legislation—A Review and a Reform," surveyed in an exceptionally clear and penetrating way the problems of international plant protection and suggested measures aiming at more effective safeguards against disease hazards with less interference with normal international commercial relations. This address was ordered printed in PHYTOPATHOLOGY.

On Wednesday afternoon a conference on coordination of plant disease research and extension was held by the Extension-Research Committee, R. J. Haskell, Chairman. Over 50 were in attendance. The discussions, led by different members of the committee, outlined definite needs for research attack on practical control problems with which the extension workers are confronted in connection with cereals, potatoes, fruit, vegetables, and ornamentals, and presented specific ways in which research and extension men can be of direct mutual assistance. The Conference voted that a committee be appointed to crystallize in the form of a resolution the strong general conviction of the necessity for regional coordination in developing disease-control programs and for regional conferences to bring this about. E. C. Stakman, J. C. Gilman, and R. H. Porter were appointed and the resolution prepared by them was adopted by the Society at the final business session. It was the sentiment of those present that another extension-research conference should be held at the next annual meeting.

On Thursday evening, a well-attended plant disease survey conference, led by H. A. Edson, discussed practical means of obtaining more systematic, more complete, more accurate, and more continuous (from year to year) records on the distribution, severity, and extent of damage from plant diseases. This was followed by a symposium on antibiosis in which M. N. Levine, G. B. Sanford, and R. Weindling analyzed the complexity of the factors involved in the suppression of plant parasitic organisms by other microorganisms. There was lively discussion afterward. Over 150 were in attendance. The Society's representatives at the Sixth International Botanical Congress at Amsterdam then presented a report and the Committee on Foreign Plant Diseases took charge of the remaining discussions of the evening, which revolved around plant protection problems and led to a

series of important resolutions, later presented to the Society and adopted at the final business meeting.

The annual phytopathological dinner was held Tuesday evening in the Statler Hotel, with almost 250 in attendance, with President H. T. Güssow as toastmaster. An interesting program, celebrating the completion of 25 years by the Society's journal, PHYTOPATHOLOGY, had been arranged by L. R. Jones with the other editors in chief assisting. Stimulating messages were read from L. R. Jones, Donald Reddick, and Perley Spaulding, who were unfortunately prevented from attending. The past achievements of the journal were discussed interestingly by H. B. Humphrey, and E. C. Stakman threw out a vigorous challenge to the membership of the Society to work together to the end that PHYTOPATHOLOGY may meet her problems successfully and serve with increasing effectiveness the field of science in the interest of which she was created. A committee of the Minnesota members of the Society arranged for a good Negro quartet and for certain special entertainment features. Twenty-five candles were lighted before the 25 volumes of the journal set in front of a painted background portraying artistically in colors some of the chief milestones in her quarter century of progress. Those present likewise greatly enjoyed the artistic vocal rendition by a group of members of a series of appropriate humorous songs introduced between the talks.

OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1936

Those whose names are followed by an asterisk (*) were elected or their appointments were confirmed by the Society at this meeting.

President, G. H. Coons*, U. S. Department of Agriculture, Washington, D. C.

Vice-President, Carl Hartley*, U. S. Department of Agriculture, Washington, D. C.

Secretary, Howard P. Barss, U. S. Department of Agriculture, Washington, D. C.

Treasurer and Business Manager of PHYTOPATHOLOGY, H. A. Edson, U. S. Department of Agriculture, Washington, D. C.

Editors, Editor in Chief of PHYTOPATHOLOGY, H. B. Humphrey, U. S. Department of Agriculture, Washington, D. C., H. M. Quanjer, Annie Rathbun Gravatt, Eubanks Carsner.

Associate Editors (3 years), T. E. Rawlins*, C. L. Lefebvre*, Edna M. Buhner*, Corabel Bien*, G. B. Sanford, A. H. Eddins, A. G. Newhall, R. P. White, F. O. Holmes, G. G. Hahn, J. J. Christensen, F. L. Drayton.

Advertising Manager of PHYTOPATHOLOGY (1 year), R. S. Kirby*, Pennsylvania State College, State College, Pa.

Councilors, N. E. Stevens* (2 years), University of Illinois, Urbana, Illinois; J. C. Walker (1 year), University of Wisconsin, Madison, Wisconsin; H. T. Güssow (1 year), Central Experimental Farm, Ottawa, Canada; V. H. Young* (1 year), University of Arkansas, Fayetteville, Ark. (representing the Southern Division); and T. E. Rawlins* (1 year), University of California, Berkeley, California (representing the Pacific Division).

PHYTOPATHOLOGICAL CLASSICS: *Editor*, H. B. Humphrey; *Business Manager*, H. H. Whetzel.

Representatives on the A. A. A. S. Council (1 year), N. E. Stevens*, H. S. Cunningham*.

Representative, Division of Biology and Agriculture, National Research Council, Howard P. Barss.

Representatives on Board of Governors, Crop Protection Institute (3 years), J. F. Adams, W. H. Martin, C. R. Orton*.

Representative on the Tropical Research Foundation (5 years), L. R. Jones*.

Representative on Subsection of Phytopathology, International Union of Biological Sciences, Donald Reddick*.

Representative on the Board of Editors, American Journal of Botany, G. W. Keitt*.

Committee on Foreign Plant Diseases, C. R. Orton, H. T. Güssow, J. S. Boyce, W. A. McCubbin, R. D. Rands.

Committee on Extension and Research, Chas. Chupp, R. J. Haskell, A. L. Pierstorff, R. S. Kirby, E. C. Stakman*, G. W. Keitt*, W. B. Tisdale*, I. L. Connors*.

Committee on Permanent Endowment, E. C. Stakman, J. G. Brown, H. H. Whetzel, A. J. Riker, L. R. Hesler.

Committee on Investments, H. A. Edson*, N. E. Stevens*, Chas. Brooks, F. C. Meier*, J. W. Roberts*.

Necrology Committee, A. G. Johnson, G. P. Clinton, M. B. Waite.

Committee on Coordination in Potato Disease Research, J. G. Leach*, J. C. Walker*, R. W. Goss*, D. Reddick*.

Committee on Coordination in Seed Treatment Research, C. S. Reddy*, W. E. Brentzel*, M. B. Moore*, H. A. Rodenhiser*.

Committee on Publication Problems (to be appointed).

TEMPORARY COMMITTEES APPOINTED IN 1935

Committee on Elections (appointed by President Güssow for the St. Louis Meeting), N. J. Giddings, L. H. Leonian.

Auditing Committee (appointed as above), R. J. Haskell, N. E. Stevens.

Committee on Resolutions (appointed as above), L. W. Durrell, V. H. Young, E. E. Clayton.

Official Committee to Represent the Society before the International Union of Biological Societies and the Sixth International Botanical Congress, Amsterdam, September, 1935 (appointed by the Council), H. T. Güssow, Donald Reddick, N. E. Stevens; alternates, J. C. Walker, E. C. Stakman.

REPORT OF THE SECRETARY FOR 1935

The Secretary has performed the usual duties of the Office and in this work has been greatly helped by the former Secretary-Treasurer, F. C. Meier, and by Mrs. Meier, who has continued to handle the membership records, journal mailing list, and other business with efficiency and dispatch. The other officers and members of the Council have participated actively in the work of the Society; and the President for the year 1935, H. T. Güssow, devoted an unusual amount of time and attention to constructive effort in the interest of the purposes for which the organization exists. Appreciation also must be expressed for the democratic way in which various members of the Society have showed their interest in its work by sending in to the Secretary at various times useful suggestions and criticisms for the consideration of the Council. It is hoped that this excellent practice may become traditional with the Society, as it will promote progress and maintain effectiveness.

Membership. The Society started the year 1935 with 798 members and ended it with 832, a net gain of 34. Sixty-two new members were elected at the St. Louis meeting, the largest group in recent years, and 12 former members were restored to the active roll during the year. The Society lost 40 members during the year, 13 by resignation, 3 by death, and 24 by suspension for non-payment of dues. Of the full membership, 107 are paid-up life members and 60 are still paying \$10 per year toward life membership. New applications for this type of membership are no longer being accepted by the Society, in view of action taken at the Atlantic City meeting in 1932.

HOWARD P. BARSS, *Secretary*.

REPORT OF THE TREASURER

Statement of Accounts for the Year Ending November 30, 1935

Receipts:

Balance from 1934		\$1,687.16
Annual dues:		
1931	\$ 5.00	
1932	10.00	
1933	20.00	
1934	50.00 (\$ 20.00, life)	
1935	2,447.50 (360.00, life)	
1936	1,646.20 (350.00, life)	
1937	20.00 (20.00, life)	
1938	20.00 (20.00, life)	\$4,218.70
Sales included in check for dues		0.60
Lyman Fund included in check for dues		2.00
Total receipts		<u>4,221.30</u>
		\$5,908.46

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY		
1934 and earlier	\$ 60.00	
1935	2,628.82	\$2,688.82
Transferred to Sinking Fund (Savings Account and Building and Loan)		330.00
Secretarial work for Treasurer, Secretary and Business Manager		354.14
Printing (programs, ballots, etc.) and mimeographing		162.10
Stamps and envelopes		68.99
Supplies		15.90
Telephone and telegraph		17.29
Expenses of Secretary-Treasurer, Pittsburgh meeting		56.26
Service charge by bank		5.30
Federal tax on checks		0.22
Total expenditures		<u>\$3,699.02</u>
Balance on hand, November 30, 1935		2,209.44
		<u>\$5,908.46</u>

Sinking Fund. At the close of 1934 the Sinking Fund, obtained by deducting \$5.00 (formerly \$6.00) from each \$10.00 life-membership installment, totaled \$8,321.00. On July 1, 1935, this amount had increased to \$8,486.00, of which \$5,000.00 was invested in first mortgage notes at 6% and \$3,486 was available for investment. A greater part of this latter amount was at that time drawing interest of 1% in a savings account, and a small part (\$305.00) had not been transferred from the checking account of the Society, where it drew no interest. Later in the year, an additional \$25.00 accrued from 1935 life-sustaining dues, making a total of \$3,511.00 available for investment during the year. It was desirable that this amount be invested with a view to both a higher rate of interest and safety for the Society's funds. In consultation with the investment committee the Treasurer invested \$3,500.00 with building associations, and the balance of \$11.00 has been deposited in a savings account.

The total interest from the Sinking Fund is available for publication of PHYTO-PATHOLOGY. During 1935 this amounted to \$472.50.

The Lyman Memorial Fund for the permanent endowment of PHYTOPATHOLOGY

On hand Jan. 1, 1935	\$1,945.95
Contributions to the fund during the year	180.29
Interest and dividends accumulated during the year (including \$47.73 interest of Jan. 1, 1936)	97.29

Total on hand Jan. 1, 1936	\$2,223.53
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(Note: At the St. Louis meeting contributions and pledges to the Lyman Fund from the Minnesota group amounting to \$150 were announced.)

H. A. EDSON, *Treasurer.*

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY

Statement of Accounts for the Year Ending November 30, 1935

Receipts:

Balance from 1934		\$1,246.82
Subscriptions:		
1933	\$ 7.25	
1934	56.60	
1935	3,054.78	
1936	468.49	\$3,587.12
Member subscriptions, 1934 and earlier		60.00
Member subscriptions, 1935		2,628.82
Sales of back numbers		339.94
Advertising:		
1934	\$209.85	
1935	564.65	774.50
Interest on 1st mortgage notes		300.00
Interest on Sinking Fund Savings Account		56.51
Classics included in check for sales		2.00
Insurance premium refunds		25.54
To replace check returned by bank		6.00
Total receipts		7,780.43
		<u>\$9,027.25</u>

Expenditures:

Printing and Distributing PHYTOPATHOLOGY:

Vol. XXIV, No. 11	\$663.22	
No. 12	395.24	
Index to Vol. XXIV	325.17	\$1,383.63
Vol. XXV, No. 1	763.83	
No. 2	676.21	
No. 3	561.97	
No. 4	477.45	
No. 5	387.61	
No. 6	562.45	
No. 7	305.63	

No. 8	430.01	
No. 9	478.80	
No. 10	193.39	
No. 11	391.65	5,229.00
Postage	646.66	\$7,259.29
List of members of American Phytopath. Soc.	146.00	
Preprints of abstracts	49.46	
Secretarial work for Business Manager	105.73	
Secretarial work for Editor in Chief	409.17	
Expenses of office of Editor in Chief	47.50	
Secretarial work for Advertising Manager	18.00	
Commission and expenses of Advertising Manager	129.84	
Stamps and envelopes	97.66	
Supplies	10.65	
Storage of back volumes	48.00	
Transfer, back volumes U. of Md. to Lancaster	11.47	
Printing (subscription bills)	21.00	
Use of car, trip to Lancaster of Business Manager and Editor in Chief	12.50	
Labor, special sale of 1933, 1934	6.82	
Telegram40	
Classics, transferred to H. H. Whetzel	2.00	
Phytopathology distribution	1.07	
Check returned by bank	6.00	
Service charge by bank	1.45	
Federal tax on checks08	
Collection charge on check35	
Total expenditures		\$8,384.44
Balance on hand, Nov. 30, 1935		642.81
		<hr/> \$9,027.25

Non-member Subscriptions. At the end of 1934 there were 541 non-member subscribers to PHYTOPATHOLOGY, including 7 complimentary subscriptions. During the year 1935 there were 23 cancellations and 24 suspensions for non-payment, a loss of 47, but with 74 new subscriptions the net gain was 27, increasing the list at the close of 1935 to 568. It is interesting to note that only 175 of these are domestic, while we have 393 foreign subscriptions on our list. Japan leads with 57, the U. S. S. R. has 42, England 35, and Canada 27.

H. A. EDSON, *Business Manager.*

REPORT OF THE AUDITING COMMITTEE

In auditing the accounts for the year ending November 30, 1935, the books of the *Treasurer of the Society* and the *Business Manager of Phytopathology* have been examined and the receipt and expenditure vouchers compared with the entries. All receipts are supported by the bank deposit slips and all expenditures are supported by receipted bills or cancelled checks, usually by both. We find the books in excellent order and believe them to be correct.

In addition, we have reviewed the investments of the *Sinking Fund* and find them to be as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection	\$5,000
Invested with the following building and loan associations:	
Northwestern Savings & Loan, at 5%	1,000
National Permanent Building Association, 5%	500
District Building and Loan, 5%	1,000
Columbia Permanent Building Association, 5%	500
Perpetual Building Association, 4½%	500
Deposited in Savings Account with McLachlen Bnk. Corporation	11
	<hr/>
	\$8,511

We also have reviewed the investment of the *Lyman Memorial Fund*, which is on deposit in the Brookland Building Association and amounts to \$2,175.80.

December 28, 1935.

NEIL E. STEVENS,
R. J. HASKELL.

REPORT OF THE EDITOR IN CHIEF OF PHYTOPATHOLOGY

The 25th volume of PHYTOPATHOLOGY, issued in 1935, contains 1,118 pages of printed text and illustrations (266 less than Volume 24) classified as follows: Eighty articles, 35 phytopathological notes, 3 reports of regional or other meetings, 6 book reviews, 169 abstracts (4 by title only), and 152 text figures, and 5 plates. During the period January 1 to December 31, inclusive, approximately 148 manuscripts of articles, phytopathological notes, reports, book reviews, etc., and 167 abstracts (4 by title only) were submitted. Of this number, 10 major manuscripts and 2 minor articles were returned to their authors for revision, 2 were withdrawn by their authors. Of the manuscripts submitted in 1934, but too late for publication that year, 3 were returned in the current year for revision. In addition to the papers published this year, 23 articles, 2 phytopathological notes, 1 national report, and 108 abstracts (1 by title only) are now, December 31, in press. The index for Volume 25 was published as a supplement to the June, 1935, number of PHYTOPATHOLOGY. The list of members of The American Phytopathological Society was published as a supplement to the May, 1935, number of PHYTOPATHOLOGY, and the Constitution of The American Phytopathological Society was published in the April, 1935, number.

A subject foremost in our thought concerning our journal relates directly to its adequate financial support. Since this will come up for detailed consideration later, I shall here merely observe that, unless our printing budget be increased by as much as \$300.00 per month, we cannot hope to keep abreast of the steady stream of acceptable manuscripts that reach the editor's desk, once the present waiting list is reduced to a working minimum.

There is now on hand a sheaf of manuscripts of sufficient volume to fill 900 pages of our journal, and acceptable papers continue to fall into the editorial hopper at the rate of one manuscript every 2½ days; and this, notwithstanding the fact that the interval between acceptance and publication is now 10 months.

During 1935 there were published in PHYTOPATHOLOGY 13 papers, or a total of 200 pages, submitted by contributors connected with a single institution. The entire cost of printing these 13 papers was borne by that institution and, in accordance with duly

authorized editorial policy, the papers were published out of the chronological order as determined by date of acceptance.

Contrary to a misapprehension entertained by some, the printing of such publication-paid manuscripts has not and will not in the least affect the amount of space assignable to any and all others, nor the promptness with which their contributions will appear.

There has been, in general, a marked improvement in the quality of all manuscripts submitted during the current year. There is, however, room for further improvement, particularly in the matter of reducing word volume to proportions reasonably commensurate with the importance of the contribution to our knowledge of the subject of which the manuscript treats. More thought should be given to this detail than has yet been given.

H. B. HUMPHREY, *Editor in Chief.*

REPORT OF THE ADVERTISING MANAGER OF PHYTOPATHOLOGY

Fifteen concerns used PHYTOPATHOLOGY for advertising merchandise in 1935. The income of \$811.59 was an 18.9 per cent decrease from that of 1934, but a 44.5 per cent increase over the advertising income of 1933.

The total number of advertisements printed was 139, of which 91 or 65.6 per cent were revenue-producing and occupied 55½ pages. Forty-eight advertisements occupying 36½ pages were non-revenue producing. These consisted of exchanges with other journals, space occupied by the directory of advertisers, announcements of meetings, and Phytopathological Classics notices.

R. S. KIRBY, *Advertising Manager.*

REPORT ON PHYTOPATHOLOGICAL CLASSICS

Report for the Fiscal Year from December 15, 1934, to December 16, 1935

Number of Classic No. 1 on hand 12-15-34	389
Total number sold	162
Balance on hand 12-16-35	227
Number of Classic No. 2 on hand 12-15-34	588
Total number sold	161
Balance on hand 12-16-35	427
Number of Classic No. 3 on hand 12-15-34	792
Total number sold	237
Balance on hand 12-16-35	555
Number of Classic No. 4 on hand 12-15-34	1020
Total number sold	381
Number gratis	15
Total number disposed of	396
Balance on hand 12-16-35	624
Cash balance on hand 12-15-34	\$207.51
Total receipts from sales for year	518.25
Total cash income	\$725.76

Expenditures:

Printing of Classic No. 4	\$282.83
Postage	37.90
Printing of cards, stationery, etc.	22.85

Total expenditures \$343.58

Balance on hand 12-16-35 \$382.18

Balance due on account 20.75

H. H. WHETZEL, *Business Manager of Phytopathological Classics.*

REPORT OF THE COMMITTEE ON ELECTIONS

Nearly 400 ballots were received. G. H. Coons was elected President, Carl Hartley was elected Vice-president, N. E. Stevens was elected Councilor for 1936 and 1937. The Pacific Division, at its last meeting, continued T. E. Rawlins as its representative on the Council for 1936. The Southern Division, at its last annual meeting, continued V. H. Young as its representative on the Council for 1936.

N. J. GIDDINGS,
L. H. LEONIAN.

REPORT OF THE COMMITTEE ON RESOLUTIONS

To the American Association for the Advancement of Science, The American Phytopathological Society wishes to express thanks for the general arrangements for the St. Louis meeting and also to C. W. Dodge, of Washington University, and his associates for working out the many details.

To the Management of the Hotel Statler, the Society wishes to express its appreciation of the many courtesies extended during these meetings.

To E. C. Stakman, and J. J. Christensen, and the members of the double quartette, the Society wishes to express appreciation for the excellent entertainment furnished at the annual banquet.

The Society also desires to thank the staff of PHYTOPATHOLOGY and the officers of the Society for their work and guidance during the past year.

L. W. DURRELL, *Chairman*,
V. H. YOUNG,
E. E. CLAYTON.

REPORT OF COMMITTEE ON NECROLOGY

During the calendar year 1935, there have been three deaths as follows:

Mr. Kinya Sasaki, March 3, 1935;
Professor Samuel Henry Essary, May 1, 1935;
Professor James M. Van Hook, June 21, 1935.

A. G. JOHNSON, *Chairman*,
G. P. CLINTON,
M. B. WAITE.

REPORT OF A. A. A. S. COUNCIL REPRESENTATIVES

Your representatives on the Council of the American Association for the Advancement of Science would report that at the St. Louis meeting the Council adopted the following resolutions of special interest to the Society: (1) Appeal for Permanent Support for International Biological Abstracts, (2) Endorsement of a Resolution Adopted at the

Sixth International Botanical Congress on the Control of Plant Diseases and Insect Pests, and (3) Endorsement of Efforts to Control Plant Diseases and Injurious Insects of Foreign Origin, Especially the Dutch Elm Disease. The latter two resolutions were based on recommendations to the Council by the Society. The full text of these resolutions appears in *Science* 83: 118-119, February 7, 1936.

N. E. STEVENS and H. S. CUNNINGHAM, *Representatives*.

DIVISION OF BIOLOGY AND AGRICULTURE, NATIONAL RESEARCH COUNCIL

Your representative attended the annual meeting on April 20, 1935. Of the items considered, the one of chief interest to the Society was the problem of the continuance of International Biological Abstracts, in view of the announced withdrawal of support by the Rockefeller Foundation. A conference called December 9 and 10, 1935, to consider the problems of abstracting and documentation of scientific literature also was attended. A resolution was adopted asking the National Research Council to set up a standing committee to consider the problem of effective support of needed abstracting and indexing agencies and calling attention to the danger to research of the discontinuance of existing agencies.

On recommendation of the Division of Biology and Agriculture, based on representations by this Society and other American biological societies, the National Research Council on August 2, 1935, dispatched a communication expressing the desire of the Council to adhere to the International Union of Biological Sciences. This action made possible the affiliation of the American Phytopathological Society to the appropriate subsection of the International Union.

HOWARD P. BARSS, *Representative*.

REPORT OF REPRESENTATIVES ON BOARD OF GOVERNORS, CROP PROTECTION INSTITUTE

A summary of projects and activities for the current year is submitted for your consideration. During the year 1935 the Board of Governors consisted of the following personnel: W. C. O'Kane, Chairman, Durham, New Hampshire; W. P. Flint, Urbana, Illinois; C. H. Richardson, Ames, Iowa; W. H. Martin, New Brunswick, New Jersey; I. E. Melhus, Ames, Iowa; J. F. Adams, Vice-chairman, Newark, Delaware; H. J. Patterson, College Park, Maryland; W. H. MacIntire, Knoxville, Tennessee, and R. Kellog, Washington, D. C., representing The American Association of Economic Entomologists, The American Phytopathological Society, The Association of Official Agricultural Chemists, and The National Research Council, respectively.

A total of 15 research projects were conducted on either a part or full-time basis of which 3 were brought to a close during the year. The headquarters for research projects or field work conducted in relation to the same were established in the following States: New Jersey, Pennsylvania, Illinois, Iowa, Florida, Delaware, Ohio, California, Connecticut, Massachusetts, Maryland, Virginia, Michigan, Colorado, Missouri, Oregon, Washington, Indiana, New Hampshire, Louisiana, New York, North Carolina, and South Carolina.

Research projects conducted during the year involved the following studies: Developing new copper fungicides, copper sulphate as a soil nutrient and plant amendment, relation of copper pots and copper lining for greenhouse benches in propagation of plants, propagation of pyrethrum, incorporation of nicotine or nicotine compounds in oil sprays, synthetic organic compounds as insecticides, various chemical compounds as fungicides, new fumigants (Proxate), incorporation of Halowax oil in petroleum oils, sulphuric acid

in weed control, utilizing carbon disulphid as a constituent of sprays, incorporation of pine oil in cattle sprays, improvement of codling moth sprays with newer forms of arsenicals, improving various forms of toxicants for insecticidal sprays, fungicidal studies with cuprous oxide and investigation of new materials for fungicidal and insecticidal sprays.

Full or part-time projects during 1935 were sponsored by the following companies: Nichols Copper Company; Stanco Incorporated, Moncanto Chemical Company, Dow Chemical Company, Liquid Carbonic Corporation, Halowax Corporation, Stauffer Chemical Company, Hercules Powder Company, General Chemical Company, Rohm and Hass Company, General Dyestuff Corporation, Metals Refining Company, A. G. Kay and J. C. Makepeace.

Publications during the year in the institute series included the following:

Bulletin No. 49, The Toxicity of Carbon Dioxide-Methyl Formate Mixtures to the Confused Flour Beetle (*Tribelium confusum* Duv.), by R. M. Jones.

Bulletin No. 50, A Method for Comparing the Ovicidal Properties of Contact Insecticides, by E. P. Breakey and A. C. Miller.

Bulletin No. 51, Halowax as an Ovicide, by E. P. Breakey.

I. E. MELHUS,

W. H. MARTIN,

J. F. ADAMS.

THE AMSTERDAM MEETINGS

Report of the Official Representative Committee of the Society, Sixth International Botanical Congress and International Union of Biological Sciences, September, 1935, Amsterdam.

(Note: About 40 members of the Society were in attendance at Amsterdam, approximately two-thirds coming from the United States. Canada was represented by four and other foreign countries by at least twelve members. H. P. B.)

International Union of Biological Sciences. Adherence of the United States to this International Union was made possible by the action of the National Research Council in the summer of 1935. It fell to members of your committee to cast the votes of the United States at the Amsterdam meeting on September 1, due to the fact that no other American societies had arranged for accredited representatives.

An American botanist, Doctor E. D. Merrill of Harvard University, was elected President of the Union.

A Subsection of Phytopathology was authorized in the Botanical Section. Doctor Donald Reddick, Cornell University, was elected Vice-President of the Botanical Section.

Several other subsections were authorized including a Subsection of Applied Botany. The latter was authorized with the distinct understanding that it should not include phytopathology. Your representatives voted against the organization of this subsection and also against the organization of a Subsection on Forestry, which was defeated.

Sixth International Botanical Congress. Members of your committee attended all sessions of the Subsection on Nomenclature, including the special sessions held by the Mycologists, as well as all sessions of the Section on Phytopathology. The results of all official actions taken at the various meetings will be published elsewhere in detail.

The actions of chief interest to this Society were:

(1) GENERAL RESOLUTION.

It is proposed that the Botanical Section of the International Union of Biological Sciences should act as an administrative connecting link between the successive International Botanical Congresses, which maintain their full independence from an international

point of view to such an extent that any country that has not joined the Union, will have quite the same rights as those that have joined.

It is proposed that this Botanical Section be authorized to carry through any resolutions, carried by the International Botanical Congresses.

Proposed by A. C. SEWARD and seconded by J. C. SCHONTE, E. J. BUTLER, E. D. MERRILL, N. NEMEC, M. J. SIRKS and N. E. SVEDELIUS.

(12) *SECTIONS, MYCOLOGY AND PATHOLOGY.*

The Sixth International Botanical Congress wishes to express its sincere appreciation of the admirable work accomplished by the Centraalbureau voor Schimmelcultures at Baarn since its foundation in 1906 and views with grave concern the present financial difficulties of what is essentially an international institution.

Proposed to the Section on Mycology by J. RAMSBOTTOM and seconded by E. J. BUTLER; to the Section on Pathology proposed by E. RHIEM and seconded by E. C. STAKMAN.

(13) *SECTION, PATHOLOGY.*

The Committee on Description and Nomenclature of Plant Viruses appointed by the Fifth International Botanical Congress 1930 wishes to report that it has made progress in developing a scheme for the nomenclature of plant viruses and suggests to this Congress that the Committee be empowered to continue its consideration and establish an acceptable system of virus nomenclature.

Proposed by H. M. QUANJER and seconded by JAMES JOHNSON, P. A. MURPHY, J. HENDERSON SMITH and G. SAMUEL.

(14) *SECTION, PATHOLOGY.*

It is recommended that the term "physiologic race" be substituted for "physiologic form," as the former seems more appropriate. It is recommended further that the word "race" be used in general to designate biotypes or groups of biotypes that differ from each other in physiologic characters.

Proposed by E. C. STAKMAN and seconded by Miss M. NEWTON, Miss G. MILBRINK and TH. ROEMER.

(15) *SECTION, PATHOLOGY.*

The Sixth International Botanical Congress expresses itself of the opinion:

that an effective and unceasing campaign against destructive plant diseases and insect pests can be successfully prosecuted only by international action and mutual cooperation;

that close and frequent international discussion of the problems of plant quarantines should take place to bring about improvement of the health conditions of plants and plant products offered for export;

that it unanimously recognizes that such action will greatly facilitate international trade in the commodities concerned, and

that, finally, this resolution be brought to the attention of the League of Nations, emphatically endorsing the League's proposal to give this matter urgent and careful consideration with a view to facilitating and expediting the purpose and aims of this resolution.

Proposed by H. T. GÜSSOW and seconded by N. VAN POETEREN.

The Seventh International Botanical Congress will be held in Sweden in 1940.

H. T. GÜSSOW, *Chairman*,
N. E. STEVENS,
J. C. WALKER.

REPORT OF COMMITTEE ON POTATO IMPROVEMENT

This committee, authorized at the St. Paul summer meeting of the Society on June 25, 1935, has studied the question of potato improvement and has reached the following conclusions:

1. That the production of disease-resistant varieties of potatoes offers one of the most promising means of controlling some of the most destructive potato diseases.

2. That the production of disease-resistant potato varieties, suitable in other respects, can most effectively be accomplished through a national cooperative potato-improvement program such as has been initiated by the office of Fruit and Vegetable Crops and Diseases of the United States Department of Agriculture and several State agricultural experiment stations.

3. That the members of The American Phytopathological Society as a group have a distinct responsibility to insure that the opportunities for potato-disease control offered by this program are utilized to the best advantage; that they should cooperate in every way possible and assume active leadership in the phytopathological aspects of the problem.

4. That the importance and promise of the national cooperative potato-improvement program is sufficient to justify its expansion with increased financial support.

To bring this matter to the attention of the proper administrative officers and technical workers, the committee prepared a short statement of the possibilities of potato-disease control through potato improvement and the need of a more adequate support of the national cooperative program. This was sent to the experiment station directors, to the Secretary of Agriculture, certain other interested administrative officers, and to the plant pathologist of each State station.

The committee has endeavored to encourage plant pathologists to take a more active part in the national program. At the St. Louis meeting of the Potato Association of America, the chairman presented a paper entitled "What the Plant Pathologist Can and Should Contribute to the Potato Improvement Program." The paper will be published in the American Potato Journal.

J. G. LEACH, *Chairman.*

REPORT OF SEED TREATMENT COORDINATING COMMITTEE

At the St. Paul summer meeting of the Society on June 25, 1935, a conference on seed treatment investigations resulted in the authorization of a committee on coordination in cereal seed treatment studies. It is the aim of this committee to encourage more uniform methods in experimentation and the recording of data, to stimulate coordination by arranging for a summer conference of the workers, to promote exchange of ideas, to find means of supplying the workers with the results of coordinated research, and to develop, on the basis of these, acceptable current control recommendations, which could be submitted for publication in PHYTOPATHOLOGY.

C. S. REDDY, *Chairman.*

REPORT ON THE TOBACCO DISEASE COUNCIL

Representatives of States interested in the production of tobacco and of the United States Department of Agriculture, including 16 members of the Society, met in conference at Greensboro, North Carolina, November 6 and 7, 1935, for the purpose of coordinating research work on diseases of tobacco. An organization designated as the Tobacco Disease Council was formed. An Executive Committee consisting of S. A. Wingard, Chairman, E. E. Clayton, R. F. Poole, W. D. Valleau, and G. M. Armstrong was named. Annual meetings of the Council will be held.

At the Greensboro conference, an extension discussion took place on various tobacco disease problems, ways and means of coordinating the efforts of the various workers, and the possibility of a cooperative regional attack on these problems. A study of the ecological relations affecting soil-borne plant parasites was listed as an important problem for a coordinated regional attack. Mimeographed copies of the proceedings of this conference were distributed. Research project outlines will be exchanged.

The Executive Committee will sponsor the organization of regional committees for the consideration of each major disease problem. These committees will summarize the data obtained from experimental work and make a report to the Council at the annual meeting.

A meeting of the Executive Committee was held January 2, 1936, in St. Louis.

R. G. HENDERSON, *Secretary*.

ACTION OF THE SOCIETY

Appointments. All appointments made by the President or Council since the previous meeting were ratified by action of the Society. These appear in the list of officers, representatives, and committees presented earlier in this report.

Reports of Officers and Committees. The various reports presented at the meeting, including that of the Resolutions Committee, were accepted by action of the Society. They appear earlier in this report.

Assistance to the Editor. On recommendation of the Council, it was voted to authorize the Editor-in-chief of PHYTOPATHOLOGY to make expenditures during 1936 up to \$350 for clerical and editorial assistance as in the previous year.

Mel. T. Cook. It was voted at the Thursday morning session that the American Phytopathological Society congratulate Dr. Mel. T. Cook for his bibliographic publications on the literature on virus diseases of plants.

Federal Agency Thanked. The Society voted an expression of appreciation to Mr. Lee A. Strong, Chief of the Federal Bureau of Entomology and Plant Quarantine, and to his staff, for the courteous manner in which the resolutions of the Society passed at the Pittsburgh meeting concerning plant protection measures had been received and for the promptness and completeness with which requests by the Committee on Foreign Plant Diseases for information had been met.

Committee on Publication Problems. On recommendation of the Council it was voted to authorize the Council to appoint a committee to study and effectuate, with the approval of the Council, ways and means of meeting our increasingly complex problems of publication.

Limit on Free Illustrations. On recommendation of Council it was voted to authorize the Editorial Board to limit to two pages of half-tones or their equivalent the number of illustrations per article except where illustrations in excess thereof are paid for by the contributor.

Charge of \$1.00 per Page Authorized. The Society endorsed the decision of the Council to exercise the authority granted at the Atlantic City Meeting in 1932 and direct a levy of \$1.00 per printed page to be made on all contributions to PHYTOPATHOLOGY accepted after January 1, 1936.

Out-of-order Publication. On recommendation of Council it was voted that the Editorial Board continue during 1936, pending the development of plans looking to bringing publication up to date, the present policy of publishing out of chronological order papers paid for in full by the contributor or supporting agency.

Interest from the Lyman Memorial Fund. On recommendation of Council it was voted that the Treasurer be authorized to allocate to PHYTOPATHOLOGY future interest from the Lyman Memorial Fund.

Life Memberships Discontinued. On recommendation of Council it was voted that Article III, Section 2 of the Constitution, providing for life members and patrons be held in abeyance pending the submission of the membership of an amendment abolishing this section.

Publication of Abstracts Discontinued but Distribution Provided for. On recommendation of Council it was voted to discontinue publication in PHYTOPATHOLOGY abstracts of papers presented at meetings of the Society and its Divisions. It was voted by the Society that abstracts of papers presented at the annual meeting of the Society be mimeographed or printed (whichever would be cheaper) at the expense of the contributors and mailed to each member of the Society in advance of the meeting.

Time Limit on Papers. On recommendation of Council it was voted that no papers presented at the annual meetings, except invitation papers, should exceed 15 minutes in length.

Place of Next Annual Meeting. It was moved by N. E. Stevens and carried that the Council be empowered to arrange for the place of the next annual meeting.

Seal of the Society Authorized. It was voted on recommendation of the Council that the Council be empowered to select an official seal for the Society.

Phytopathological Classics. It was voted to authorize the management of Phytopathological Classics to circularize the membership for advance subscriptions which would insure sufficient income, when added to funds already on hand, to permit the publication of the translations in this series, now nearly ready.

Resolution on Regional Coordination in Plant Disease Research and Control Programs. This resolution and that following were adopted by the Society on recommendation of the Extension-Research Conference.

It is the sense of this meeting that the American Phytopathological Society should formulate plant disease control programs after careful consideration by extension and research men. To this end it is desirable to have committees study individual problems and make recommendations at joint meetings of extension and research workers at the annual meetings. It also is desirable to stimulate coordinated regional effort through encouragement of regional meetings and cooperative endeavor in formulation of regional research and extension programs, and, when desirable and practicable, in the formulation of national problems.

Resolution on Coordination of Research and Extension Effort. It has become evident in recent years and as a result of discussions at joint extension and research conferences at the annual meetings that research and extension work would be far more effective if it were possible for extension and research men to cooperate more closely.

It is resolved, therefore, that this meeting recommend that The American Phytopathological Society call to the attention of National and State research and extension directors that urgent need for closer coordination of effort by research and extension workers.

Resolution on International Cooperation in Plant Protection. It was moved by H. W. Anderson and carried that the following resolution passed by the Sixth International Botanical Congress be endorsed by the Society and transmitted to the American Association for the Advancement of Science and the National Research Council and other interested agencies.

Resolved: "That an effective and unceasing campaign against destructive plant diseases and insect pests can be successfully prosecuted only by international action and mutual cooperation;

that close and frequent international discussion of the problems of plant quarantines should take place to bring about improvement of the health conditions of plants and plant products offered for export;

that it unanimously recognizes that such action will greatly facilitate international trade in the commodities concerned, and

that, finally, this resolution be brought to the attention of the League of Nations, emphatically endorsing the League's proposal to give this matter urgent and careful consideration with a view to facilitating and expediting the purpose and aims of this resolution."

Dutch Elm Disease Resolution. On recommendation of Council the Society passed the following resolution:

Resolved: That The American Phytopathological Society recommend to the Council of the American Association for the Advancement of Science that it strongly endorse the efforts of Federal and State Agencies to control the Dutch elm disease, and, furthermore, that the Council of the American Association for the Advancement of Science be respectfully requested to take such measures as may be necessary to bring to the attention of the proper authorities the desirability of continued and adequate support of the Dutch elm disease control project.

The following six resolutions were prepared by the Committee on Foreign Plant Diseases, in accordance with instructions by the Society, and adopted by vote of the Society at the Wednesday evening conference.

Resolution on Need for More Adequate Plant Detention Services. Resolved: That in view of the great danger of releasing imports before complete freedom from dangerous plant diseases can be assured, more adequate detention services be established, and that this resolution be communicated by the Committee on Foreign Plant Diseases to the Secretary of Agriculture, the Chief of the Bureau of Entomology and Plant Quarantine, and other officials concerned with the administration of these services.

Resolution on Study of Foreign Plant Diseases Abroad. Resolved: That the policy of sending specialists into foreign countries to study potentially dangerous plant diseases be continued and expanded, and that this matter be brought before the proper authorities by your committee.

Resolution on Foreign Biotypes of Plant Pathogens. Resolved: That the same caution be taken with respect to the introduction of biological races of dangerous plant parasites as is considered necessary in the case of specific entities.

Resolution on Import of Living Cultures of Plant Pathogens Under Permit. Resolved: That in view of the potential dangers of the unrestricted entry of living cultures of plant pathogens, the Society recommends that such cultures be permitted import only on permit. At the same time the Society is cognizant of the need of living cultures for scientific purposes and recommends as free entry of such cultures as is consistent with adequate safeguards in the national interests.

Resolution on Extension of Elm Disease Survey. Resolved: That the present policy for extending search for the Dutch Elm Disease is inadequate and that the Society urges the extension of survey work into all territory where elms are grown and where there may be a possibility of this disease having become established.

Resolution on Feasibility of Potato Wart Eradication. Resolved: That a further statement of evidence on the feasibility of carrying out eradication methods in case of the Potato Wart Disease be prepared and presented to the Chief of the Bureau of Entomology and Plant Quarantine.

SAMUEL HENRY ESSARY

SEPTEMBER 11, 1870—APRIL 28, 1935

Samuel Henry Essary was graduated from the University of Tennessee in 1897 and received the degree of Master of Science from the same institution in 1907. From 1899 to 1902, he was instructor in science in La Grange College, La Grange, Missouri, and from 1902 to 1904 he taught science in Brenau College, Gainesville, Georgia. In 1904 he was appointed instructor in botany, University of Tennessee, and assistant botanist in the Tennessee Agricultural Experiment Station. Subsequently, he was promoted successively to associate botanist and botanist of the Experiment Station, which latter position he held to the time of his death.

He was an active member of a number of scientific and social organizations and was a charter member of The American Phytopathological Society. He did notable work in developing anthracnose-resistant clover and wilt-resistant tomatoes. He was an enthusiastic naturalist and loved the "Great Out-of-doors" and all it contains: the natural scenic beauty as well as the native flora and fauna, which he knew so well in his area. While he did no formal teaching, particularly in his later years, yet he was a great inspiration to the younger botanists, with whom he loved to associate. In the words of one of these " . . . his greatest pleasure was to see the science for which he had devoted his own life advanced by the accomplishments of those younger men in whom he was especially interested."

HERMAN JOHN NINMAN

MAY 4, 1876—OCTOBER 24, 1934

Herman John Ninman was graduated from the University of Wisconsin in 1913 with the degree of Bachelor of Science in Agriculture, and in 1917 he received the degree of Master of Science from the same institution.

From 1914 to 1915, Mr. Ninman was principal of the Tripoli High School, Tripoli, Wisconsin. From February 1 to April 30, 1917, he was employed by the Federal Bureau of Plant Industry at Madison, Wis., as assistant in tobacco disease investigations. During the following summer he was employed as a member of a white pine blister rust scouting crew in northwestern Wisconsin. During the same fall he served for a time as nursery inspector in the office of the State Entomologist of Wisconsin. From December 18, 1918, to the time of his death, he served almost continuously as Agent on the rolls of the U. S. Department of Agriculture in various capacities in connection with white pine blister rust activities in Wisconsin.

Mr. Ninman was a most earnest, highly dependable worker, always working with patience and unusual tenacity. While he was very unassuming in his attitude, he was always ready to encourage a worthy cause and to help its advancement in any way he could.

FRANK LINCOLN STEVENS

APRIL 1, 1871—AUGUST 18, 1934

Frank Lincoln Stevens was graduated from Hobart College, Geneva, N. Y., in 1891 and received the degrees of Bachelor of Science and Master of Science from Rutgers University in 1893 and 1897, respectively. In 1900 he received the degree of Doctor of Philosophy from the University of Chicago. In 1925 he was given the honorary degree of Doctor of Science by San Marcos University, Peru, and in 1931 he received the honorary degree of Doctor of Laws from the University of Glasgow.

During 1898 and 1899, Dr. Stevens served as analyst for the Chicago Drainage Commission while he was pursuing graduate study at the University of Chicago. In 1901 he served as instructor in biology at the North Carolina State College, and in 1902 he became professor of botany and vegetable pathology in the same institution, which position he held until 1912. From 1903 to 1912, he also was biologist for the North Carolina Agricultural Experiment Station. From 1912 to 1914 he served as dean of the College of Agriculture, University of Puerto Rico. From 1914 to the time of his death, except for temporary absences, he served as professor of plant pathology in the department of botany at the University of Illinois. During 1921-1922, he held the Bishop Museum Fellowship from Yale and spent the year collecting fungi in the Hawaiian Islands; in 1924-1925 he made an extensive collecting trip in South America; and in 1930-1931 he held the Baker Memorial Professorship of Botany at the University of the Philippines. He was active in a number of scientific societies and was a charter member of The American Phytopathological Society and its second president.

Dr. Stevens was the author of numerous books and scientific papers. He was a man of broad interests in the field of biology. He had unusual foresight, was a very keen observer, tireless collector, inspiring teacher, able investigator, and ready writer. He was always highly considerate of others, ever congenial, yet dignified. It has been truly said of him that "his keen sense of right and his firm but calm and kindly spirit endeared him to all who worked with him."

JAMES M. VAN HOOK

DECEMBER 19, 1870—JUNE 20, 1935

James M. Van Hook was graduated from Indiana University in 1899 with Bachelor of Arts degree and received the degree of Master of Arts from the same institution in 1900. During 1900-1901 he was assistant in botany, Cornell University, and from 1902-1904, he was assistant in plant pathology in the Extension Department of Cornell University. From 1904 to 1907 he was assistant botanist at the Ohio Agricultural Experiment Station. In 1907 he was appointed assistant professor of botany, Indiana University, and subsequently was promoted successively to associate professor and to full professorship in 1925, which position he held to the time of his death.

Professor Van Hook was active in a number of scientific societies and was a charter member of The American Phytopathological Society. He was an able investigator and a very capable, enthusiastic teacher. He contributed much to our knowledge of the cause and control of certain diseases of ginseng and of peas and added greatly to our knowledge of the fungus flora of Indiana. It is said of him by one of his associates that: "His friendships were deep and lasting. Those who knew him recognized and admired his integrity, sincerity, kindness, and loyalty; and were impressed with his knowledge, skill, and ability in the field of botanical science, which he loved."

PHYTOPATHOLOGICAL SUMMER MEETING

IOWA STATE COLLEGE, AMES, IOWA
JUNE 25-26, INCLUSIVE

First Day

- 9:00 A.M.: Registration, Botany Hall, Room 202.
Tour through Laboratory and Greenhouses.
1:00 P.M.: Tour of Phytopathological Plots, Agronomy Farm,
Horticultural Orchards, and Federal Erosion
Nursery.
6:30 P.M.: Dinner, Memorial Union.
8:00 P.M.: Extension Workers' Round-table Discussion.
Coordination of Programs.
9:00 P.M.: Round-table Discussion—Research.
Relation of Phytopathology to Erosion Control.

Second Day

- 8:00-10:30 A.M.: Research—Round-table Discussion.
Is There a Place for Regional Research Programs in
Plant Pathology?
12:30 P.M.: Dinner, Leader Cafe, Kanawha.
1:30 P.M.: Excursion to Northern Iowa Experimental Farm,
Kanawha, Iowa—Cereals, Beets, Potatoes, etc.
Excursion to Southeastern Experimental Farm,
Conesville, Iowa—Melons, Sweet Potatoes, Other
Truck Crops.
Excursion to Western Iowa Fruit Section, Glenwood,
Iowa—Apple Spraying, Apple Root Rot, Nursery
Diseases.

PHYTOPATHOLOGY

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VIRUS CONCENTRATION IN RELATION TO ACQUIRED IMMUNITY FROM TOBACCO RING SPOT¹

W. C. PRICE

(Accepted for publication February 26, 1936)

INTRODUCTION

In 1932, the writer (5) described recovery of several species of *Nicotiana* from tobacco ring spot. It was shown that this recovery is a normal behavior of plants infected with the disease, that it occurs under most environmental conditions, and that it is distinguished from masking of symptoms, which occurs only under exceptional environmental conditions. It was, furthermore, shown that recovered plants cannot be induced to develop symptoms by reinoculation with virus from diseased plants. This phenomenon of recovery and subsequent failure to develop symptoms on reinoculation was referred to as acquired immunity. It differs from acquired immunity from most animal diseases in that it is not accompanied by complete disappearance of virus. Because of the retention of virus by recovered plants, several workers, notably Smith (7), Köhler (4), and Valteau (10), have objected to the use of the term acquired immunity in this connection. Smith prefers to consider the phenomenon as acquired immunity from the clinical picture of ring spot or as acquired tolerance. Köhler, likewise, prefers the term acquired tolerance. Valteau, on the other hand, maintains that the so-called recovery is nothing more than a stage of the disease; that the plants do not actually recover from the disease, but merely develop patternless leaves in which the cells are parasitized to the limit.

In order to obtain a better understanding of the nature of this phenomenon, a study was made of the relative virus concentration in various parts of recovered and diseased tobacco plants. The results furnish additional evidence that here, indeed, is a non-sterile immunity comparable with that that obtains in a number of virus diseases of animals.

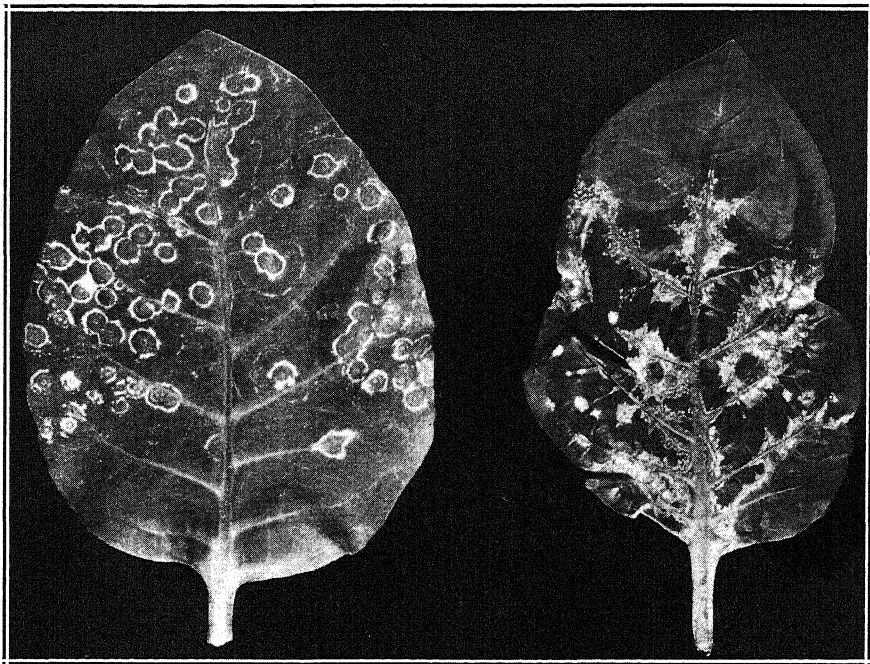
DESCRIPTION OF THE DISEASE

A detailed description of tobacco ring spot has been given by Wingard (11) and by Price (5). Only a brief review of the characteristic symptoms

¹ Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

produced in tobacco (*Nicotiana tabacum* L. var. Turkish) as different stages of the disease develop will be presented.

Transmission of the disease is readily obtained by the rubbing method of inoculation. Primary lesions develop in the inoculated leaves within 3 or 4 days. They consist of rings or circles of necrotic or chlorotic tissue alternating with rings of healthy-appearing tissue. They also may occur as solid necrotic spots. Figure 1 illustrates a leaf bearing necrotic primary lesions and a leaf showing systemic lesions.

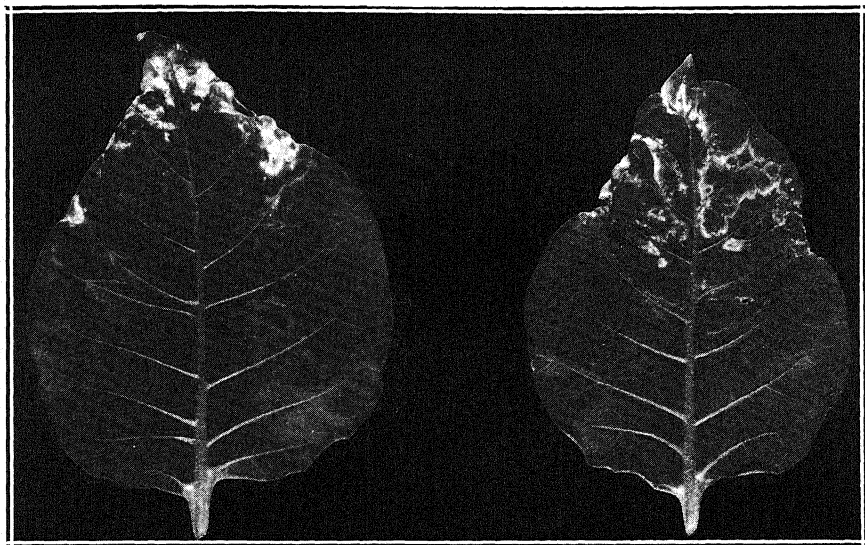


Photographed by J. A. Carlile

FIG. 1. Leaves of Turkish tobacco showing (left) primary and (right) systemic necrotic lesions produced by ring-spot virus.

Systemic lesions appear 6 or more days after inoculation. They are similar to the primary lesions, but usually are larger, especially when centered on large veins. They are also fewer in number than the primary lesions produced by rubbing with undiluted inoculum.

The third stage in the disease, and one that marks the beginning of the plant's recovery, is initiated from 10 to 14 days after inoculation. Leaves are produced that show wavy lines of chlorotic or necrotic tissue extending across their apical portions, leaving the basal portions free from necrosis and healthy in appearance (Fig. 2). The first of such leaves to develop have



Photographed by J. A. Carlile

FIG. 2. Leaves of Turkish tobacco showing the characteristic oak-leaf pattern that occurs during the process of recovery from tobacco ring spot. Such leaves are referred to as partly recovered leaves, since symptoms appear only on their apical portions.

only a small fraction of the base free from necrosis but, as new leaves continue to appear, the lines are situated closer and closer to the tips of the leaves leaving a larger and larger fraction of the basal part free of symptoms.

Finally, leaves are produced that are completely free from chlorotic and necrotic lesions and are, as a rule, indistinguishable from healthy leaves. The leaves produced after this fourth stage has been reached never develop lesions characteristic of the onset of the disease.

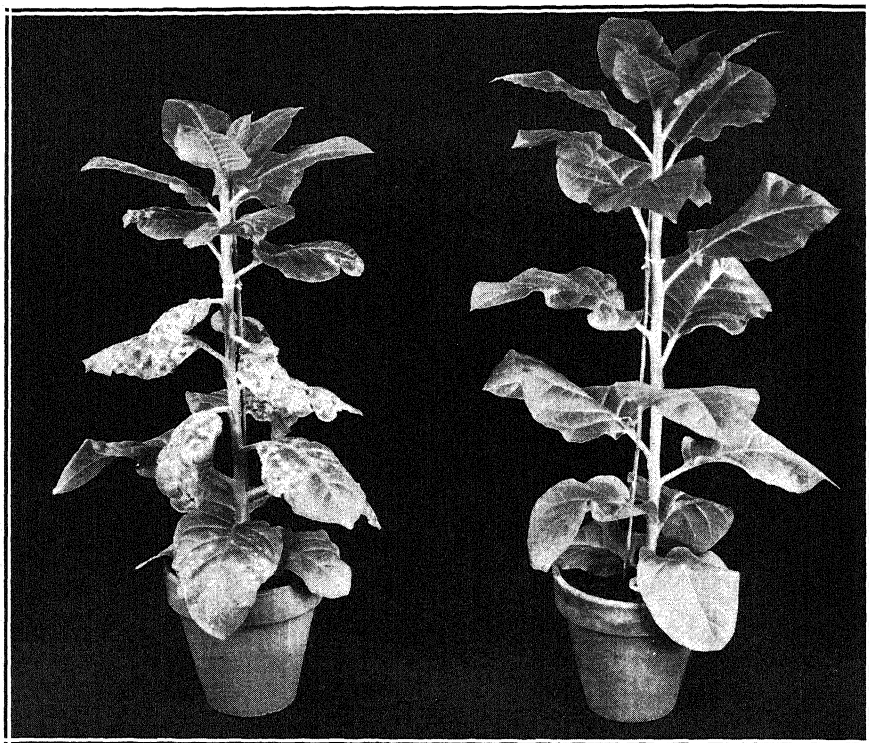
Figure 3 shows a photograph of a plant that has recovered from ring spot. It will be seen from an examination of this figure that the leaves near the tip of the plant are free from lesions and healthy in appearance and that leaves below the tip show characteristic ring-spot lesions.

For convenience, the healthy-appearing leaves on plants that have recovered from ring spot will be referred to as recovered leaves, and those bearing necrotic lesions as diseased leaves.

MATERIALS AND METHODS

Ring-spot Virus: A brief description of a disease later shown to be tobacco ring spot was given by Valteau² in 1921. A more detailed description was given by Fromme and Wingard (2, p. 16-17) in 1922. It was not, however,

² Valteau, [W. D.]. Kentucky, a disease of tobacco. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bull. 5: 107. 1921. [Mimeographed]



Photographed by J. A. Carlile

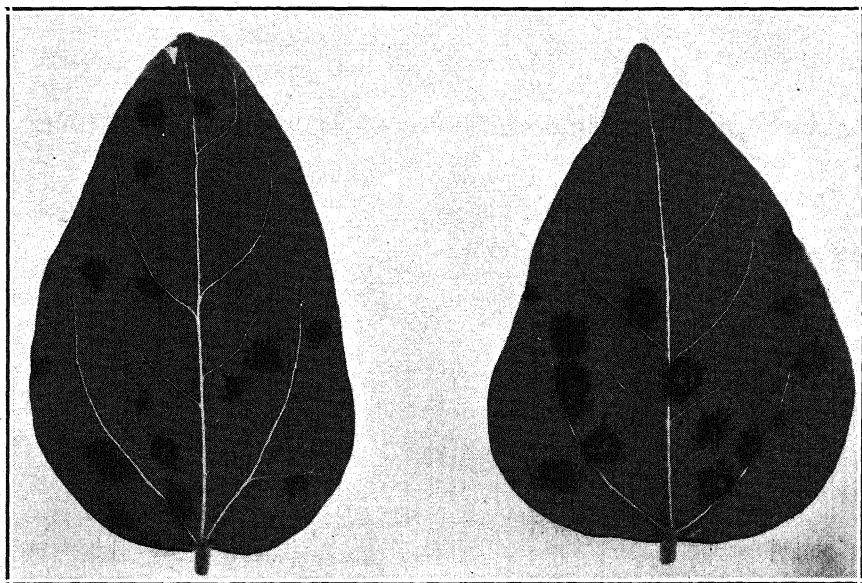
FIG. 3. A Turkish tobacco plant infected with ring spot (left) and a healthy plant of the same age (right). The infected plant has recovered from the disease and developed a number of healthy-appearing leaves at the tip. Symptoms appear only on the basal leaves, which were infected during the onset of the disease.

until the publication of a paper by Fromme, Wingard and Priode (3) in 1927 that the disease was definitely shown to be infectious, although Fromme³ reported in 1923 that Valteau had obtained 2 infections out of about 200 attempts by the rubbing method. The writer obtained the virus from Wingard in 1929 and has maintained it continuously in tobacco plants since that time. The virus does not appear to be identical with either the green or yellow ring-spot viruses described by Valteau (9), but it is likely that it may be a strain of his green ring-spot virus.

Host Plants. *Nicotiana tabacum* L. var. Turkish served as the host plant in all the studies to be reported in this paper. The plants were grown in 4- or 6-inch porous clay pots in a greenhouse. They were, as a rule, inoculated when from 2 to 4 inches in height. Inoculations were invariably made by means of the rubbing method.

³ Fromme, [F. D.]. Ring spot or hieroglyphics. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bull. Sup. 26: 142. 1923. [Mimeographed.]

Preparation of Virus Samples for Inoculation. In determining the virus concentration in different plants or in different leaves on a plant, the preparation of the virus sample was as follows: A 2-inch square of tissue was cut from the leaf to be tested, was thoroughly macerated in a sterile mortar, and was diluted with 4 cc. of water. The solution thus obtained was applied to leaves of the test plants by means of a small square of cheese cloth. In those cases in which two or more samples were taken from the same leaf, a $1\frac{1}{2}$ -inch square of tissue was used. For measurements made using stems as a source of virus, a section of stem, $1\frac{1}{2}$ to 2 inches in length, was cut from a point near the tip of the plant tested. Measurements of virus concentration in roots were made in the same manner, an approximately equal volume of root tissue being used for each test. The actual juice dilution of virus used is estimated to be approximately 1:5 to 1:10 for the leaf samples and 1:1.5 to 1:2 for the stem and root samples.



Photographed by J. A. Carlile

FIG. 4. Necrotic primary lesions produced by tobacco ring-spot virus in leaves of Black cowpea. The photograph was taken 5 days after inoculation.

Measurement of Virus Concentration. An estimate of the virus concentration in the different samples tested was secured by inoculating leaves of the Black variety of cowpea, *Vigna sinensis* Endl., and counting the numbers of lesions produced. It was found previously (5) that the numbers of lesions produced in the cowpea leaves decreased progressively as the virus content of the inoculum decreased. Figure 4 illustrates the lesions pro-

duced in this host plant 5 days after inoculation. The plan was to inoculate 40 cowpea leaves with each of the virus samples. Accordingly, the cowpeas were planted 6 to a pot, 4-inch pots being used in each instance. Five pots of cowpeas were used for each of the virus samples tested. Not all the seed germinated and grew, the numbers varying from 2 to 12 leaves (1 to 6 plants) per pot available for test. The plants were inoculated when they were from 6 to 9 days old, only the first pair of true leaves on each plant being used. These leaves had attained a size of from $\frac{1}{2}$ to $\frac{3}{4}$ their maximum at the time of inoculation. The figures given in the tables represent the number of lesions obtained for 40 cowpea leaves with each of the virus samples tested. The figures were obtained by dividing the actual number of lesions by the total number of leaves and multiplying the quotient by 40. This was done in order to eliminate fractions, and because 40 was the actual number of leaves used in many instances.

*Statistical Analysis.*⁴ The data obtained from quantitative measurements of ring-spot virus were subjected to the analysis of variance. The analysis consists essentially in the segregation of that part of the variation that is associated with specific causes and provides an estimate of the experimental error freed from the variability of known origin. A discussion of the analysis of variance is to be found in Fisher's text (1). It has been applied recently to data from plant-virus studies by Youden and Beale (12). In the present paper the values designated as significant, and those designated as highly significant, were determined by the application of Snedecor's (8) table XXXV. The table was computed by Snedecor from Fisher's table VI. The values designated as significant correspond to Fisher's 5 per cent point (odds of 20 to 1), while those designated as highly significant correspond to the 1 per cent (odds of 100 to 1).

At the outset it may be said that there are certain definite sources of variation to be taken into consideration in interpreting the data presented in the following pages. The experiments were, as a rule, designed to determine the difference in concentration between virus taken from different plants or from different parts of plants. Variation arising from this source usually accounts for a large fraction of the total. Of the other sources of variation common to all the experiments, that between tests is perhaps the most consistently significant throughout. The large value attributed to this variance emphasizes the importance of making comparisons only between measurements made at the same time and under the same environmental conditions. Probably the difference in susceptibility of cowpea plants grown under slightly different environmental conditions accounts for a large part of the variation between tests. In most cases the interaction has proved to

⁴ The writer is greatly indebted to Dr. John W. Gowen for advice and assistance in the statistical analysis of the data presented in this paper.

be significant, indicating a rather large variation in trend between the different tests in the same experiment. Another source of variation is the origin of the virus samples. The large mean square for the variation between inoculum samples in the same experiment-and-treatment subclass shows that, in nearly all cases, virus samples taken from comparable groups of plants grown under similar conditions varied considerably in concentration. It is likely that plants grown under different environmental conditions would vary even more widely in virus content. The other sources of variation to be considered are concerned with the susceptibility of cowpea plants to ring-spot virus. With one exception, plants in different pots showed a greater variation than plants in the same pot. Likewise, leaves on the same plants varied less than leaves on different plants.

EXPERIMENTAL

I. Multiplication of Virus in Recovered Plants

While this paper is concerned chiefly with virus concentration, it should not be out of place to refer here briefly to multiplication, or increase, of virus in plants that have recovered from ring spot, inasmuch as this question may have a direct bearing upon some phases of the main problem. It was found earlier (5) that recovered plants retained the virus of this disease when grown through 3 generations from cuttings. This finding was not, however, considered as conclusive evidence that the virus multiplies in recovered plants, since the treatment did not serve to dilute the virus originally present in the plant to a great enough extent. In this work the plan was to grow recovered plants through a sufficient number of generations from cuttings to insure a high dilution of the virus originally present, and then to test these plants for presence of virus. Twenty Turkish tobacco plants were inoculated with ring-spot virus and allowed to recover from the disease. When the recovered plants had attained a height of from 2 to 3 feet, tip cuttings were taken from each, rooted in a mixture of peat moss and sand, and, finally, potted in 6-inch pots. A second set of cuttings was later taken from each of the plants grown from cuttings of the first set. The process was continued until a total of 10 generations from cuttings had been grown over a period of a little more than 2 years. Occasionally, to prevent the young plants from going to seed, it was necessary to cut off the tops and allow the axillary buds to develop. Assuming an equal distribution of virus in each plant, it is estimated that, averaged on the basis of green weight, approximately $1/16$ of the virus present in the plant was retained in the cutting. Over the period covered by the experiment, the virus originally present should have attained a dilution of more than 1×10^{-12} in the 10th series of cuttings. At no time during the 2 years did necrotic or chlorotic lesions appear in the plants. They occasionally showed an inconspicuous

yellowing or necrosis along the leaf edges similar to that previously illustrated and described (5), but, as a rule, they showed neither chlorosis nor necrosis. They were, however, somewhat more stunted than plants grown from seed. Each of the 20 plants grown from cuttings was tested for ring-spot virus by inoculation of healthy Turkish tobacco plants. An appreciable amount of virus was found to be present in each of the 20 plants tested. Since infection is not ordinarily obtained in tobacco with dilutions greater than 1×10^{-4} , it is concluded that the virus must have multiplied in the recovered plants. The symptoms produced in tobacco by virus from recovered plants grown through 10 generations from cuttings were no different from those produced by virus from diseased plants. The virus was, therefore, not attenuated after 2 years in recovered plants.

II. Virus Concentration of Leaves of Recovered and Diseased Plants

An experiment was performed for the purpose of determining whether or not the virus concentration of leaves of recovered plants differs significantly from that of leaves of diseased plants. Measurements were made of virus in 10 plants that had recovered from ring spot and in 10 plants showing acute symptoms of the disease. A leaf showing numerous necrotic lesions was selected from each of the diseased plants and used as a source of inoculum. A leaf of approximately the same size and, as nearly as possible, of the same physiological age was selected from each recovered plant and likewise used as a source of inoculum. Each sample was used to inoculate 5 pots of cowpeas. The inoculations were alternated so that the first set of 5 pots was inoculated with juice from a recovered plant and the next set with juice

TABLE 1.—*Relative virus concentration of leaves from Recovered (R) and diseased (D) tobacco plants infected with ring spot as indicated by the numbers of lesions produced in 40 cowpea leaves*

Plant No.	Test No. 1		Test No. 2		Test No. 3		Test No. 4	
	R	D	R	D	R	D	R	D
1	79	484	50	295	61	83	15	283
2	45	521	17	209	25	174	21	130
3	81	555	114	410	91	107	62	237
4	161	592	74	244	31	264	34	89
5	140	387	60	386	8	46	31	81
6	50	353	50	325	19	71	43	193
7	49	430	34	688	34	123	29	232
8	118	254	82	201	142	46	67	309
9	27	650	27	232	72	227	30	283
10	39	527	42	173	9	44	16	126
Total	789	4753	550	3163	492	1185	348	1693

from a diseased plant. The experiment was repeated on 3 different occasions with 3 different sets of plants. The average numbers of lesions obtained in the 4 tests are given in table 1. The results show that the diseased leaves contained more than 5 times as much virus as recovered leaves.

The data of table 1 were subjected to the analysis of variance, the results of the analysis being shown in table 2. In this experiment, 40 cowpea leaves

TABLE 2.—*Statistical analysis of the data summarized in table 1*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves on same plants	1,600	10,797.5	6.7
Between plants in same pot	1,200	24,891.4	20.7
Between pots of same inoculum	320	10,325.6	32.3
Between inoculum samples in same test-and-treatment subclass	72	12,378.2	171.9
Between test-and-treatment subclass	7	43,048.1	6,149.7

were used for each recovered or diseased plant; 400 leaves were inoculated with virus from recovered plants and 400 with virus from diseased plants in each of the 4 tests. A total of 3200 cowpea leaves were, therefore, used. While 5 pots of cowpeas were used for each recovered or diseased plant, the number of leaves per pot, unfortunately, varied from 2 to 12 with a harmonic mean of 7.27. The unequal number of leaves per pot introduced some heterogeneity in the direct analysis of the 3 sources of variation in the between-test-and-treatment subclass; between the different tests, between virus derived from recovered or diseased plants, and for the differential effects of the virus. To obviate this difficulty, the mean numbers of lesions per leaf for each pot were used and table 3 was derived.

TABLE 3.—*Analysis of the 3 sources of variation in the between-test-and-treatment subclass of table 2*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between tests	3	1,305.6	435.2
Between virus from recovered <i>vs.</i> diseased leaves	1	2,930.1	2,930.1
Interaction	3	883.9	294.6

The appropriate variance to test the significance of the items in table 3 is that between pots of the same inoculum, 32.3, divided by the harmonic mean of the plants per pot, 7.27; or perhaps a better means of comparison is to multiply the mean squares of table 3 by 7.27 and then compare them

with those of table 2. These values are: between tests, 3163.9; between virus of recovered and diseased plants, 21,301.8; and interaction, 2141.7.

The data show that the factor in the variation of the lesions per leaf within the experiment that contributed most to the total variation was the source of the virus, whether it was obtained from a plant that had recovered or from one that was diseased. The statistical significance of the result is beyond question, since the mean square due to this cause, 21,301.8, divided by the estimated variance, 32.3, is 659.5, where 6.7 would be highly significant.

The variation due to differences in the conditions under which the 4 tests were performed has likewise resulted in a rather large effect. The significant value of the interaction indicates that the relative virulence of the recovered and diseased virus samples varied from test to test. Turning to table 2, the significantly large mean square of 171.9 for the inoculum samples in the same test-and-treatment subclass shows that the tobacco leaves varied in virus concentration.

The 2 mean squares—between leaves of the same plant and between plants in the same pot—bear on two problems of somewhat secondary importance to this experiment but of primary interest in connection with the technique of such experiments and the general factors affecting virus diseases. The small mean square between the lesions of the two leaves of the same plant treated in the same manner shows the close similarity in the reactions of these leaves to the same stimulus. In view of this fact, a better technique than that used for the experiment would have been to treat one of the leaves of the plant with the virus of the recovered plant and the other leaf with the virus of the diseased plant, and then make the comparison. In this connection it should be noted that Samuel and Bald (6) have suggested the use of the "half-leaf" method for estimation of tobacco-mosaic virus concentration and Youden and Beale (12) have suggested a Latin-square arrangement of leaves of test plants in order to minimize the variation resulting from differences between test plants.

The virus reactions on the different plants vary, showing that there is a factor innate in the test cowpea plants that tends to make them more or less resistant to the virus. These facts are evident in all subsequent experiments.

III. Virus Concentration of Healthy-appearing and Diseased Parts of Partly Recovered Leaves

In the process of recovery of a tobacco plant from the ring-spot disease, leaves are produced that develop symptoms only on their tips, as has already been mentioned. Such leaves usually show an oak-leaf pattern, consisting of one or more zigzag lines of necrotic tissue, near the middle of the leaf, and a number of zonate circular lesions near the tip. Such a leaf is illustrated in

figure 2. In this paper, the basal part of a leaf showing an oak-leaf pattern will, for convenience, be referred to as the recovered portion, the apical part as the diseased portion, and the entire leaf as a partly-recovered leaf. Two tests were conducted, in each of which measurements were made of the virus concentration in apical and basal parts of partly-recovered tobacco leaves. The results of the tests are given in table 4. The statistical analysis of the experiment is summarized in table 5. The data show that the diseased parts

TABLE 4.—*Relative virus concentration of recovered (R) and diseased (D) parts of partly recovered leaves as indicated by the average number of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 1		Test No. 2	
	R	D	R	D
1	161	520	325	464
2	262	668	201	289
3	299	425	188	224
4	147	359	129	166
5	124	276	68	90
6	208	438	236	203
7	163	289	90	155
8	114	145	117	240
9	56	205	62	192
10	64	126	120	318
Total	1598	3451	1536	2341

^a The average was multiplied by 40 to avoid fractions and because in many instances the actual number of leaves used for test was 40.

TABLE 5.—*Statistical analysis of the data summarized in table 4*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	899	10,225.5	11.4
Between plants of same pot	699	21,398.9	30.6
Between pots of same inoculum	160	9,716.2	60.7
Between inoculum samples in same test and treatment	36	12,985.7	360.7
Variance based on pot means and adjusted for the harmonic mean number of lesions per pot			
Between tests	1	837.8	837.8
Between virus from recovered vs. diseased parts of same leaves	1	4,440.2	4,440.2
Interaction	1	690.5	690.5

Harmonic mean number of leaves per pot is 8.61.

of the leaf contained considerably more virus than the recovered parts. The result is clearly significant, since the ratio of the mean squares, 4440.2/60.7 or 73.1, is much more than 3.9 for significance.

IV. Virus Concentration of Apical and Basal Parts of Recovered and of Diseased Leaves

As a check to the experiment discussed in Section III, measurements were made of the virus concentration in apical and basal parts of recovered leaves and, likewise, in apical and basal parts of diseased leaves. Ten recovered and 10 diseased leaves were used for the experiment. The diseased and recovered leaves were tested at different times. The results of this experiment are given in table 6 and an analysis of the results in table 7. The data

TABLE 6.—*Relative virus concentration of apical (A) and basal (B) parts of diseased and recovered leaves as indicated by the average numbers of lesions per leaf multiplied by 40^a*

Plant No.	Diseased leaves		Recovered leaves	
	A	B	A	B
1	849	818	326	285
2	668	721	54	88
3	454	494	60	174
4	344	222	115	191
5	537	379	186	342
6	109	250	226	224
7	394	193	232	301
8	355	407	252	256
9	493	486	226	236
10	393	709	247	209
Total	4596	4679	1924	2325

^a See footnote, table 4.

show that there was no significant difference in the virus concentration of the apical and basal parts of either the diseased or recovered leaves. As might be expected from table 1, the largest contribution to the variation is that between recovered and diseased leaves. The interaction is not significant. Referring back to Section III, the difference in virus concentration between recovered and diseased parts of partly recovered leaves cannot be ascribed to a positional effect, but must result from a dissimilarity of recovered and diseased tissues. Consequently, it may be concluded that the healthy-appearing tissues are associated with a low virus content and the necrotic tissues with a high virus content. These tissues are sharply delimited as regards both external appearance and virus concentration.

TABLE 7.—*Statistical analysis of the data summarized in table 6*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	673	13,199.3	19.6
Between plants of same pot	471	55,739.6	118.1
Between pots of same inoculum	160	23,984.7	149.9
Between inoculum samples in same test and treatment	36	19,328.2	536.9
Variance based on pot means and adjusted for the harmonic mean number of leaves per pot			
Between diseased and recovered leaves	1	12,914.9	12,914.9
Between virus from apical <i>vs.</i> basal parts of leaf	1	4.8	4.8
Interaction	1	68.7	68.7

Harmonic mean number of leaves per pot is 5.97.

V. Virus Concentration of Partly Recovered and Fully Recovered Leaves

A comparison of tables 1 and 4 has indicated that, while the virus concentration of diseased parts of partly recovered leaves is comparable with that of diseased whole leaves, the recovered parts of partly recovered leaves might have a slightly higher concentration of virus than recovered whole leaves. Two tests, therefore, were conducted in which a comparison was made between recovered parts of 10 partly recovered leaves and 10 fully recovered leaves taken from the same plants. The data are presented in table 8. Table 9 gives the statistical analysis of these data. The results

TABLE 8.—*Relative virus concentration of partly recovered (P) and fully recovered (F) leaves as indicated by the average numbers of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 1		Test No. 2	
	P	F	P	F
1	204	290	431	304
2	75	244	278	117
3	204	526	231	300
4	114	210	33	147
5	138	280	42	42
6	199	265	18	87
7	123	274	74	154
8	250	290	52	104
9	77	248	76	77
10	155	315	51	87
Total	1539	2942	1286	1419

^a See footnote, table 4.

TABLE 9.—*Statistical analysis of the data summarized in table 8*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	832	6,861.5	8.2
Between plants in same pot	632	18,202.6	28.8
Between pots of same inoculum	160	6,755.9	42.2
Between inoculum samples in same test and treatment	36	9,141.0	253.9
Variance based on pot means and adjusted for the harmonic mean number of lesions per pot			
Between tests	1	1,682.3	1,682.3
Between virus from partly recovered leaves <i>vs.</i> fully recovered leaves	1	1,336.3	1,336.3
Interaction	1	887.2	887.2

Harmonic mean number of leaves in pot is 7.81.

show that, within the 2 tests, the recovered parts of partly recovered leaves contained significantly less virus than fully recovered leaves from the same plants, the ratio of the mean squares, 1336.3/42.2, is 31.7 where 3.9 would be significant. The variation in materials as shown by the rather wide differences in the lesions of the 2 tests and the differential changes in the reaction of the virus as shown by the interaction, were both markedly significant.

The results are in conflict with those roughly obtained by a comparison of tables 1 and 4. Possibly an explanation for the significant difference in virus concentration between partly recovered and fully recovered leaves lies in the fact that the recovered leaves were, in every case, younger (taken from nearer the tip of the plant) than the partly recovered leaves. Another possible explanation is, however, the phenomenon of over-compensation frequently observed in vital processes. The tissues that are to recover may overreact to the virus causing a greater inhibition of virus multiplication than is the equilibrium value for the recovered plant. This equilibrium value may be established later in the fully recovered leaves.

VI. Virus Concentration of Inoculated Leaves of Recovered Plants, of Noninoculated Leaves of Recovered Plants, and of Inoculated Leaves of Previously Healthy Plants

Since it has been shown that recovered leaves almost invariably contain a lower concentration of virus than diseased leaves, an experiment was conducted to determine whether or not the virus content of recovered leaves could be increased by inoculating them with ring-spot virus. Healthy plants and recovered plants of as nearly the same age, size, and appearance as was possible to find were selected. The recovered plants were divided into 2

groups. One group of recovered plants, and all the healthy controls were inoculated by rubbing several leaves with ring-spot virus. The other group of recovered plants was not inoculated. Five days after inoculation, measurements were made of the virus concentration in inoculated leaves of the healthy controls, in inoculated leaves of the first group of recovered plants, and in noninoculated leaves of the second group of recovered plants. The data from 2 tests are given in table 10, and the analysis of the data in table 11. The comparison between the mean square for the virus source and the experimental error shows a highly significant difference, 24,340.7/77.8, a ratio of 312.9 to 1, where 3.9 would be significant. From the table containing the original data, it is obvious that differences in the 3 different virus treatments do not contribute equally to the variation. The estimate of the variance, 77.8, may be used to test the significance of these differences. The standard deviation is equal to $\sqrt{77.8}$, or 8.82. The total number of lesions produced

TABLE 10.—*Relative virus concentration of leaves of recovered plants (R), of reinoculated recovered plants (IR), and of inoculated healthy plants (IH), as indicated by the average numbers of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 1			Test No. 2		
	R	IR	IH	R	IR	IH
1	27	87	720	67	103	250
2	57	211	460	143	165	396
3	77	79	511	162	120	817
4	16	52	461	38	131	778
5	18	44	756	317	256	1084
6	37	100	478	18	139	753
7	61	89	297	85	48	478
8	8	37	475	139	122	360
9	72	35	331	135	184	403
10	43	35	430	190	122	129
Total	416	769	4919	1294	1390	5448

^a See footnote, table 4.

by juice from the recovered plants is 1675 on 756 leaves; by juice from reinoculated plants, 1947 on 700 leaves; and by juice from inoculated healthy plants, 9509 on 722 leaves. The mean lesions per leaf for each group are 2.22, 2.78, and 13.17. The standard errors of the differences of these results are $8.82 \sqrt{\frac{1}{756} + \frac{1}{700}} = 0.463$, $8.82 \sqrt{\frac{1}{756} + \frac{1}{722}} = 0.459$, and $8.82 \sqrt{\frac{1}{700} + \frac{1}{722}} = 0.468$, respectively. The differences between the groups are $2.78 - 2.22 = 0.56$, $13.17 - 2.22 = 10.95$, and $13.17 - 2.78 = 10.39$, leading to the values of 1.2 with 1454 degrees of freedom, 23.86 with 1476 degrees of freedom, and

TABLE 11.—*Statistical analysis of the data summarized in table 10*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	1,089	14,345.5	13.2
Between plants of same pot	789	70,778.7	89.7
Between pots of same inoculum	240	18,670.1	77.8
Between inoculum samples in same test and treatment	54	27,688.0	512.7
Variance based on pot means and adjusted for the harmonic mean number of leaves per pot			
Between tests	1	1,301.2	1,301.3
Between virus from recovered, inoculated recovered, and inoculated healthy plants	2	48,681.4	24,340.7
Interaction	2	77.8	38.9

Harmonic mean number of leaves per pot is 6.49.

TABLE 12.—*Relative virus concentration of roots of recovered (R) and diseased (D) plants as indicated by the average numbers of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 1		Test No. 2	
	R	D	R	D
1	39	125	232	324
2	43	126	125	295
3	120	157	94	141
4	143	147	90	185
5	76	92	195	388
6	67	85	106	278
7	69	64	53	132
8	43	57	101	128
9	42	52	175	83
10	15	33	82	54
Total	657	938	1253	2008

^a See footnote, table 4.

22.20 with 1420 degrees of freedom. The results consequently show that the only significant difference was that between the inoculated healthy controls and either the recovered or reinoculated recovered plants. The recovered and reinoculated recovered leaves did not differ significantly in virus content. No evidence was obtained that the virus concentration of recovered leaves may be increased by inoculating them heavily with ring-spot virus.

VII. Virus Concentration of Roots of Recovered and Diseased Plants

Since it was shown that leaves of recovered and diseased plants differ significantly in virus concentration, experiments were undertaken to test

other parts of the plants. Measurements were made on the virus concentration of roots of recovered and diseased tobacco plants. Root samples that were approximately equal in volume were selected for the tests. Unfortunately, it was necessary to use recovered plants that were about 2 weeks older than the diseased plants. The roots were washed in tap water and macerated in a mortar with 4 cc. of water. Results of 2 tests with roots of recovered and diseased plants are given in table 12. A statistical analysis of the data is given in table 13, which shows that there was a significant difference in the virus content of the roots from recovered and diseased plants. The ratio of the mean square, 645.9, to the experimental error, 16.6, is 38.9 where 3.9 would be significant. Roots of recovered plants contained, on the average, considerably less virus than roots of diseased plants. That the roots varied widely in virus concentration is shown by the ratio of the mean square between inoculum samples in same test and treatment to the experimental error, $125.4/16.6 = 7.6$. The most significant difference was, however, that between tests.

VIII. Virus Concentration of Stems of Recovered and Diseased Plants

An experiment was performed in order to determine whether or not stems of diseased plants have a higher virus concentration than stems of recovered plants. A section of stem was removed from the tip part of each of 10 recovered and 10 diseased Turkish tobacco plants. The recovered plants used for test were somewhat older than the diseased plants and consequently had slightly thicker stems. The difference in thickness was compensated for by adjusting the length of the stem section, so that a total volume of approximately 2.5 cc. was obtained. Table 14 shows the results of the measurements made with stems from 2 sets of diseased and recovered plants. The statistical analysis of the data is given in table 15. From these results no evidence was obtained that there is an essential difference in virus concentration of stems of recovered and diseased plants. The ratio between the mean square of the recovered *vs.* diseased stems and the experimental error is $34.4/13.3 = 2.6$, where the minimum value to be significant would have to be 3.9.

IX. Virus Concentration of Diseased Plants as Compared with Plants Grown through 10 Generations from Cuttings of Recovered Plants

In Section I of the paper, mention was made of the use of recovered plants grown through 10 generations from cuttings for the purpose of determining whether or not virus multiplies in recovered plants. Since these same plants were available for test, it seemed desirable to compare their virus content with that of diseased plants. Four tests were conducted in which the virus concentration of leaves of recovered plants grown through 10 generations from cuttings was compared with that of leaves showing acute symptoms of

ring spot. The results of the experiment are given in table 16 and an analysis of the data in table 17. The data show that recovered plants grown from cuttings had a considerably lower virus titer than diseased plants.

X. Virus Concentration of Recently Recovered Plants as Compared with Recovered Plants Grown through 10 Generations from Cuttings

Four tests were conducted in which measurements were made of the virus concentration of leaves from recently recovered plants and of leaves from recovered plants grown through 10 generations from cuttings. The data from these experiments are given in tables 18 and 19. They show that recovered plants in the 10th generation from cuttings contain considerably less virus than recently recovered plants. The ratio of the mean squares is 222.1 where 3.9 would be significant. The interaction is not significant.

The recovered plants obtained from cuttings appeared to grow less rapidly and were somewhat more stunted than the recently recovered plants. It is possible that the stunting may account for at least a part of the pronounced difference in virus concentration of the two groups of plants.

XI. Virus Concentration of Inoculated and Systemically Diseased Leaves

Three tests were conducted to determine whether or not inoculated leaves contain a higher virus concentration than systemically diseased leaves. In each test, measurements were made of the virus concentration in 10 inoculated and 10 systemically diseased leaves. The results of these experiments are presented in table 20. Table 21 gives the statistical analysis of the data.

The results show that the inoculated leaves in general contained more virus than systemically diseased leaves. The difference is clearly significant, the mean square $10,653.2/173.7 = 61.3$ where 6.7 would be highly significant. Examination of table 20 reveals that the inoculated leaves had consistently more virus than the systemically diseased leaves in both tests 1 and 3, whereas test 2 is anomalous in showing 8 out of 10 systemically diseased leaves with a larger virus concentration than their inoculated mates. In this connection it is well to consider in detail the methods employed in collecting the data. In test 1, each pair of inoculated and systemically diseased leaves was taken from the same infected tobacco plant. In test 2, the leaves making up a pair were taken from different plants and an effort was made to select systemically diseased leaves that showed as many necrotic lesions as shown by the inoculated member of the leaf pair. In the 3rd test, the leaves were from different plants and the samples of inoculum were prepared as follows: A large-size cork borer (number 6) was used to cut out a section of leaf tissue including a necrotic lesion. As a rule, only one lesion was removed with each disc of tissue, but in a number of instances more than one lesion was included. Ten such discs of tissue were cut from each of the leaves tested. This procedure was followed in an attempt to include the same number of necrotic

TABLE 13.—*Statistical analysis of the data summarized in table 12*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	795	2,753.0	3.5
Between plants of same pot	595	6,935.9	11.7
Between pots of same inoculum	160	2,663.9	16.6
Between inoculum samples in same test and treatment	36	4,516.0	125.4
Variance based on pot means and adjusted for the harmonic mean number of lesions per pot			
Between tests	1	1,559.0	1,559.0
Between virus from recovered vs. diseased roots	1	645.9	645.9
Interaction	1	113.0	113.0

Harmonic mean number of leaves per pot is 7.34.

TABLE 14.—*Relative virus concentration of stems of recovered (R) and diseased (D) plants as indicated by the average numbers of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 1		Test no. 2	
	R	D	R	D
1	71	163	67	137
2	104	85	64	67
3	67	57	15	75
4	194	19	78	84
5	126	73	53	111
6	111	68	96	87
7	51	76	79	117
8	25	65	204	160
9	118	90	61	148
10	47	66	40	172
Total	914	762	757	1158

^a See footnote, table 4.

lesions in each inoculum sample. The total quantity of leaf material used in test 3, therefore, was considerably less than that used in either test 1 or 2. This difference in quantity of leaf tissue accounts, in part, for the large variation between tests and also is reflected in the large value attributed to the interaction.

The mean difference in lesions per leaf between inoculated and systemically diseased leaves in test 1 is $12.44 \pm .7927$ (where $\pm .7927$ is equal to the standard error of this difference); in test 2 it is $-1.75 \pm .8829$; and in test 3 it is $1.70 \pm .3723$. The difference is highly significant in test 1, where the

TABLE 15.—*Statistical analysis of the data summarized in table 14*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	810	2,812.0	3.5
Between plants in same pot	610	4,890.5	8.0
Between pots of same inoculum	160	2,121.9	13.3
Between inoculum samples in same test- and-treatment subclass	36	1,759.3	48.9
Variance based on pot means and adjusted for the harmonic mean number of leaves per pot			
Between tests	1	34.4	34.4
Between virus from recovered <i>vs.</i> diseased stems	1	34.4	34.4
Interaction	1	202.2	202.2

Harmonic mean number of leaves per pot is 8.83.

tobacco leaves were selected at random; it is not significant in test 2 and just barely significant in test 3, where selection was made on the basis of the numbers of necrotic lesions present in the tobacco leaves tested.

The data derived from the above experiment lead to the conclusion that inoculated leaves contain more virus on the average than do systemically diseased leaves and that this difference is, for the most part, accounted for by the difference in numbers of lesions to be found in inoculated and systemically diseased leaves. The effect of numbers of lesions on virus concentration is considered in more detail in Section XII.

XII. Virus Concentration of Leaves Inoculated with Different Dilutions of Ring-spot Virus

Two experiments were performed for the purpose of determining the effect of numbers of ring-spot lesions on virus concentration. The plan of the experiment was to determine the virus concentration of tobacco leaves inoculated with different dilutions of ring-spot virus. Five leaves of Turkish tobacco were inoculated with undiluted ring-spot virus, and 5 with virus diluted 1:5, 1:25, and 1:125. After 5 days an estimate was made of the numbers of lesions present in each of the 4 sets of leaves. The total numbers of lesions obtained were 1125 for the undiluted virus sample, 447 for the 1:5 dilution, 163 for the 1:25 dilution, and 36 for the 1:125 dilution. Measurements were then made of the virus concentration in each of the 4 sets of leaves. The experiment was repeated about a month later, but the numbers of lesions produced in the inoculated tobacco leaves were not recorded. Table 22 shows the numbers of lesions that were obtained from the 2 tests. An analysis of the data is given in table 23. The results show that the larger

the number of lesions present in a tobacco leaf, the greater, in general, is the virus concentration of that leaf.

The values for virus concentration are quite consistent in the 2 experiments, as indicated by the lower proportion of the variation contributed by the interaction. Considerable of the variation between leaves of diseased tobacco plants may, therefore, be attributed to the evident differences between the numbers of lesions present on such leaves.

DISCUSSION

By far the greatest contribution to the variation within the experiments reported in this paper is that between tests. This large difference results principally from the variation in susceptibility of the separate lots of cowpeas used as test plants and only partly from a variation in virus concentration of different lots of tobacco plants affected by ring-spot virus. That the latter do show considerable variation in virus concentration is demonstrated by the differences between inoculum samples within the same test-and-treatment subclass.

The evidence shows conclusively that recovery of tobacco plants from ring-spot disease is accompanied by a marked decrease in virus concentration. It is tentatively estimated that recovered leaves contain from 1/5 to 1/10 as much as actively-diseased leaves. The cells of recovered plants are not, as Valleau (10) has suggested, parasitized to the same limit as the cells of diseased plants.

TABLE 16.—*Relative virus concentration of leaves of recovered plants grown through 10 generations from cuttings (C) and recently diseased plants (D), as indicated by the numbers of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 1		Test No. 2		Test No. 3		Test No. 4	
	C	D	C	D	C	D	C	D
1	82	448	106	467	96	754	118	241
2	99	525	51	602	102	657	27	211
3	205	500	75	427	116	649	30	411
4	205	565	51	289	242	756	83	346
5	239	951	19	304	103	640	59	185
6	236	422	67	300	183	1023	196	355
7	9	195	72	158	140	511	92	386
8	302	570	35	378	252	885	211	387
9	24	359	76	203	127	762	58	447
10	45	272	230	152	202	573	178	289
Total	1446	4807	782	3280	1563	7210	1052	3258

^a See footnote, table 4.

The difference in virus concentration between recovered and diseased plants affords a partial explanation for acquired immunity from tobacco ring

TABLE 17.—*Statistical analysis of the data summarized in table 16*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	1,641	24,837.5	15.1
Between plants of same pot	1,241	93,565.1	75.4
Between pots of same inoculum	320	33,406.0	104.4
Between inoculum samples in same test and treatment	72	21,976.4	305.2
Variance based on pot means and adjusted for the harmonic mean number of leaves per pot			
Between tests	3	17,355.3	5,785.1
Between virus from diseased plants <i>vs.</i> plants grown from cuttings	1	55,277.6	55,277.6
Interaction	3	8,341.2	2,780.4

Harmonic mean number of leaves per pot is 7.76.

TABLE 18.—*Relative virus concentration of leaves of recovered plants grown through 10 generations from cuttings (C) and recently recovered plants (R), as indicated by the average numbers of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 1		Test No. 2		Test No. 3		Test No. 4	
	C	R	C	R	C	R	C	R
1	65	194	103	212	4	83	60	69
2	36	155	99	318	77	194	65	95
3	65	149	91	179	41	214	30	59
4	19	175	22	228	91	179	36	61
5	73	174	42	320	4	122	54	151
6	91	277	84	266	78	138	36	130
7	98	197	167	282	39	145	68	123
8	198	171	101	407	111	84	106	213
9	19	197	413	175	93	70	111	76
10	62	207	32	453	51	225	69	113
Total	726	1896	1154	2840	589	1454	635	1090

^a See footnote, table 4.

spot. It does not, however, explain the mechanism for this phenomenon. That there must be a definite mechanism for regulating the multiplication of virus in recovered plants seems beyond question. Whether this mechanism involves the action of antibodies in the immune plants, or whether it is dependent on something else, is at present unknown. An hypothesis for re-

TABLE 19.—*Statistical analysis of the data summarized in table 18*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves of same plants	1,565	8,474.0	5.4
Between plants of same pot	1,165	17,064.0	14.6
Between pots of same inoculum	320	6,991.1	21.8
Between inoculum samples in same test and treatment	72	7,135.8	99.1
Variance based on pot means and adjusted for the harmonic mean number of lesions per pot.			
Between tests	3	3,359.7	1,119.9
Between recently recovered plants and plants grown from cuttings of recovered plants	1	4,841.9	4,841.9
Interaction	3	987.0	329.0

Harmonic mean number of leaves per pot is 7.23.

covery and acquired immunity from tobacco ring spot is as follows: The ring-spot virus is able to reach its maximum concentration in, and to exert its maximum effect upon, only those cells that are nearly mature at the time of invasion. The invasion of embryonic cells of tobacco plants is not accompanied by maximum increase in virus nor by severe injury to the cells. Having once been invaded, the embryonic cells become adapted to the presence of virus and maintain an equilibrium value with the virus. Symptoms are produced in tobacco plants only so long as the growing point has not been

TABLE 20.—*Relative virus concentration of inoculated (I) and systemically diseased (S) leaves as indicated by the average numbers of lesions per leaf multiplied by 40^a*

Plant No.	Test No. 3		Test No. 2		Test No. 1	
	I	S	I	S	I	S
1	422	232	726	815	376	268
2	612	404	905	998	265	211
3	973	241	971	610	417	361
4	940	551	626	715	451	359
5	928	341	722	754	345	305
6	999	270	897	1307	384	283
7	756	524	895	1054	160	110
8	814	415	1009	1223	295	166
9	605	136	950	656	182	173
10	1471	374	651	878	165	88
Total	8520	3488	8352	9010	3040	2324

^a See footnote, table 4.

TABLE 21.—*Statistical analysis of the data summarized in table 20*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves on same plants	1,253	31,290.5	25.0
Between plants in same pot	953	149,815.8	157.2
Between pots of same inoculum	240	41,683.0	173.7
Between inoculum samples in same test and treatment	54	46,238.2	856.3
Variance based on pot means and adjusted for the harmonic mean number of lesions per pot.			
Between tests	2	87,052.7	43,526.4
Between virus from inoculated leaves <i>vs.</i> systemically diseased leaves	1	10,653.2	10,653.2
Interaction	2	20,594.4	10,297.2

Harmonic mean number of leaves per pot is 7.91.

invaded. Once the embryonic cells become infected the plant recovers. The hypothesis is supported by the fact that the course of the disease may be prolonged or shortened by exposure of infected plants to good or poor growing conditions (5). Rapidly growing plants tend to grow away from the virus and required a longer time for recovery. When very young plants are inoculated, the virus rapidly reaches the tip of the plants and they quickly

TABLE 22.—*Relative virus concentration of leaves inoculated 5 days previously with certain dilutions of ring-spot virus, as indicated by the average numbers of lesions per leaf multiplied by 40^a*

	Plant no.	1: 125 Dilution	1: 25 Dilution	1: 5 Dilution	Undiluted
Test 1	1	107	353	563	425
	2	142	407	341	664
	3	124	93	937	821
	4	230	517	799	587
	5	146	188	583	723
	Total	749	2358	3223	3220
Test 2	1	31	202	295	145
	2	103	236	285	409
	3	96	89	278	307
	4	9	74	222	178
	5	25	120	326	585
	Total	264	721	1406	1624

^a See footnote, table 4.

TABLE 23.—*Statistical analysis of the data summarized in table 22*

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Between leaves on same plants	746	13,271.5	17.8
Between plants in same pot	546	55,370.5	101.4
Between pots of same inoculum	160	22,469.9	140.4
Between inoculum samples in same test and treatment	32	17,509.0	547.2
Variance based on pot means and adjusted for the harmonic mean number of lesions per pot.			
Between tests	1	14,266.3	14,266.3
Between virus concentrations	3	18,803.7	6,267.9
Interaction	3	2,038.2	679.4

Harmonic mean number of leaves per pot is 6.70.

recover from the disease. An objection to acceptance of the hypothesis is the fact that there is no definite proof that the embryonic cells at the growing point are ever invaded by virus.

The demonstration that virus multiplies in recovered plants raises the interesting question of where multiplication takes place. Ring spot is essentially a spot disease. It is well known that virus increase occurs in the primary lesions, and it has been assumed that increase also occurs in systemic lesions. The primary and systemic lesions are generally regarded as marking the areas of virus multiplication. No visible lesions occur in recovered plants, but virus has been found to multiply in these plants. There is at the present time little evidence regarding the locus of this multiplication. One possibility is that increase occurs in definite spots not marked by symptoms, and that virus moves from these spots into the growing point of the plant. Another possibility is that virus multiplies in the embryonic cells of the growing point of recovered plants, and that it moves into each new cell that is developed. The cells of the growing point may be capable of restricting the multiplication of virus to a level that is not sufficient to cause the production of lesions.

It is of interest that the decrease in virus concentration is confined to the leaves and roots, and that stems of recovered and diseased plants do not differ significantly in virus titer. The latter is not surprising, since stems never show symptoms of the disease. On the other hand, it is difficult to explain the difference between roots of recovered and diseased plants, since the roots have not been observed to show definite symptoms of ring spot. Perhaps the fact that, in the experiments reported, the roots of diseased plants were younger than those of recovered plants might account for the difference in virus concentration.

It is believed that recovery of tobacco plants from the ring-spot disease, accompanied by a marked decrease in virus concentration, and followed by immunity from reinfection, constitutes an acquired immunity comparable in many respects to that that obtains in virus diseases of animals.

SUMMARY

1. By growing recovered plants through a series of 10 generations by means of cuttings and subsequently testing for virus, it was shown that ring-spot virus multiplies in tobacco plants that have recovered from the ring-spot disease.

2. Using the number of necrotic primary lesions produced in leaves of Black cowpea (*Vigna sinensis* Endl.) as a measure of virus concentration, it was shown that leaves from diseased plants contain, on the average, from 5 to 10 times as much virus as leaves from recovered plants. The leaves of both recovered and diseased plants were found to vary considerably in virus concentration.

3. Measurements made of virus concentration in partly recovered leaves of tobacco plants affected by ring spot have shown that the healthy-appearing (basal) portions contain considerably less virus than the diseased (apical) portions of the same leaves. No evidence was obtained that apical and basal portions of either diseased or recovered leaves differ in virus concentration.

4. Fully recovered leaves were found to contain more virus than the healthy-appearing portions of partly recovered leaves.

5. The virus content of recovered leaves was not shown to have been increased by inoculating them heavily with ring-spot virus, although healthy leaves of the same age that were inoculated at the same time showed approximately 5 times as much virus as either the inoculated recovered or noninoculated recovered leaves tested 5 days after inoculation.

6. The roots of diseased plants were found to contain significantly more virus than the roots of recovered plants.

7. No evidence was obtained that there is an essential difference in virus concentration in stems of recovered and diseased plants.

8. Recovered plants grown through 10 generations from cuttings were found to contain much less virus than diseased plants. They were, likewise, found to contain somewhat less virus than recently recovered plants.

9. Leaves inoculated with undiluted virus contain, as a rule, more virus than systematically diseased leaves. This difference is partly accounted for by the numbers of necrotic lesions present in such leaves.

10. Measurements made 5 days after inoculation of tobacco leaves with various dilutions of ring-spot virus showed that the larger the number of lesions present in an inoculated leaf the greater, in general, is the virus content of that leaf.

11. Cowpea plants used for testing virus concentration were found to vary considerably in susceptibility to ring-spot virus, the variation being greater in plants grown under different conditions than in those grown under the same conditions. Variation between leaves on the same plants was found to be less than that between leaves on different plants.

FROM THE DEPARTMENT OF ANIMAL AND PLANT
PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR
MEDICAL RESEARCH, PRINCETON, NEW JERSEY

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BLACK ROT OF TOMATO, *LYCOPERSICUM ESCULENTUM*, CAUSED BY *ALTERNARIA* SP.¹

ELSBETH E. WARNER²

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INTRODUCTION

In the black rot of tomato the spots either appear on the outside and progress toward the center or they appear in the center and progress toward the outside, frequently without showing any signs of unsoundness on the surface. Investigation revealed that the rot was caused by a fungus, *Alternaria* sp. Although various species of *Alternaria* have been studied by others, this investigation has brought to light some unexpected characteristics in the life history of the species of *Alternaria* causing this disease, and some results have been obtained that may indicate the method of infection.

HISTORICAL REVIEW

Douglas (1) found that the *alternaria* disease of tomato appeared after the first fall rains and generally increased until the end of the season, and secured infection by placing drops of spore suspensions "on well-sized, green fruits just previous to the time they began to change color." Characteristic *alternaria* spots also were produced on the leaves of some varieties of tomato by placing them in a moist chamber and spraying them with a suspension of the spores in sterile water. The leaves of certain varieties did not yield to this method of infection.

Gibson (2) reported *Alternaria atrans*, severely infecting soy beans and cow peas, following injury by sunburn and aphid bites.

Guba (3) found that the viability of spores of *Alternaria dianthi* was destroyed after 2 years in the open field.

Martin (4) reports that *Alternaria solani* spores were carried, not only on the bodies but also in the excreta of 4 different species of insects that customarily visit the tomato. Many spores were found also on the hands and garments of pickers.

Pool (5, p. 3-6) studied a disease of tomato caused by *Alternaria fasciculata*, which displayed symptoms similar to those of the disease here discussed, although the fungus which she describes differs widely from that of the present discussion.

Whetzel (6) found severe infection on potatoes caused by *Alternaria* sp. following a rainy season.

¹ This work was done in partial fulfillment of the requirements for the Master of Arts degree in Plant Pathology, in Oberlin College.

² The writer acknowledges her indebtedness to Dr. S. P. Nichols and Prof. George T. Jones for their valuable advice and criticism during the course of the investigation.

MATERIALS AND CONDITIONS

The present investigation was begun in the open field during the growing season of 1934, in connection with other experiments on tomato. The summer was very hot and dry, making frequent cultivation necessary for the conservation of the soil moisture. None of the plants was watered artificially. They were completely surrounded by corn except for a distance of about 2 rods where peas, peppers, and bush beans were planted in adjacent space. The competition of the corn for the scant water supply doubtless contributed to the dryness of the soil.

The tomato vines were carefully staked to protect the fruit from contact with the soil. In this connection it should be noted that the tomatoes in surrounding or near-by gardens were not staked, the fruit being allowed to lie on the ground. In these gardens the infection was much more severe than in the plot where the experiment was being conducted and where the disease was rather infrequent.

The prevailing winds were S.W. to N.W. and N.E., and it is interesting here to note that the tomatoes on the north end of the garden were most severely infected. Attention should be called to the fact that at this end of the garden the corn was so stunted as to be hardly taller than the tomato plants themselves.

Varieties used in the field were Marglobe, Ponderosa, and Pritchard. Heaviest infection appeared on the Marglobe, but its greater susceptibility to the disease has not been definitely established. In previous years other varieties planted in the same area always showed some infection.

SYMPTOMS

Infection occurs on both green and ripe fruit, and is confined to the fruit. The infection spots are not restricted to one location on the fruit, but are more generally found externally at the blossom end (Figs. 1 and 2, A). The infected areas are slightly wrinkled, dark brown, and, in their later stages, develop velvety mats of conidiospores, as observed by Douglas (1). The spots range in size from minute pinheads to areas extending completely across the surface of the fruit, giving it a flattened face. After the diseased fruit had been separated from the vine and exposed to dampness a grayish white mycelial growth appeared on the lesions rapidly spreading over the entire fruit (Fig. 2). If the infected areas are internal with no evidence of the disease on the surface of the fruit, the spots range from 1 to 2 inches in diameter and are pitch black. This internal rot is dry and leathery. The underlying tissue of the more severely infected portions of the spots is extremely tough, and is, therefore, very difficult to section. Free-hand sections, made with a safety-razor blade, showed that the most recently infected

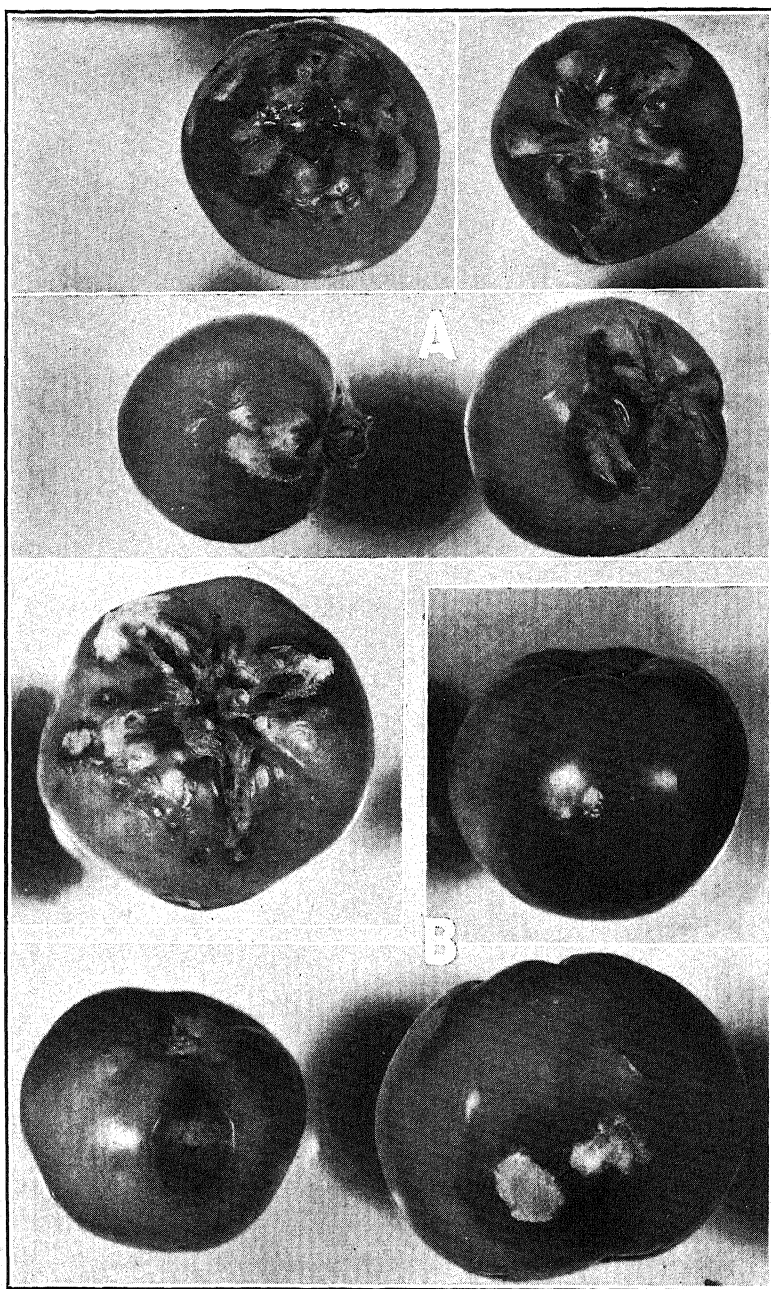


FIG. 1. A. Stem-end infections, resulting from inoculations through mechanical wounds, while the fruit was still on the vine. B. Lateral infection resulting from stab while fruits were still on the vine.

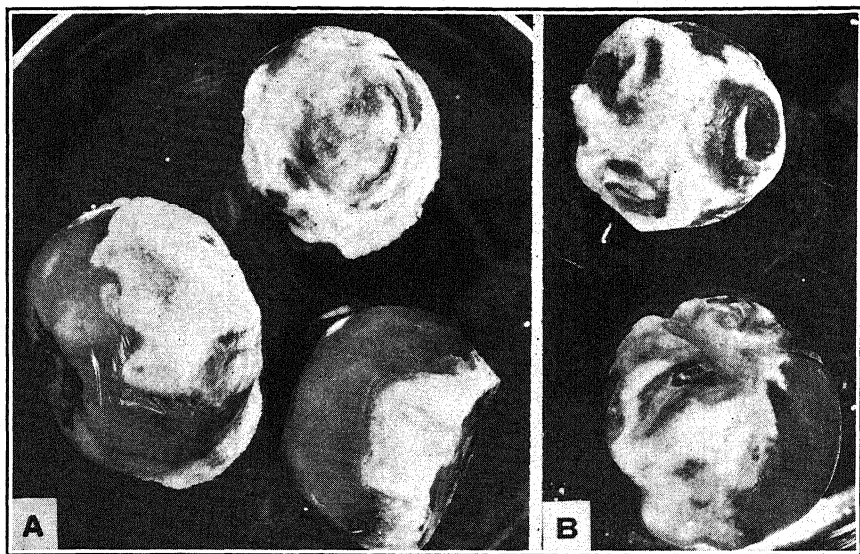


FIG. 2. A. Blossom-end infection. Infection developed on the vine and fruits were later placed in moist chamber, bringing out the gray mycelial growth over the infected areas. B. Mechanical injuries produced on fruit off the vine. Fruits kept in moist chamber, resulting in mycelial growth.

tissues were tan to brown,³ while the region of severest infection was dark brown to black. Microscopic examination revealed a dense mass of dark brown hyphae in the older areas, with brownish to green to almost hyaline filaments penetrating the surrounding healthy tissues. These symptoms differ from those published by Pool (5) who found the hyphae to range from black in the older areas to brownish in the areas of least infection.

In order to make microtome sections of the tomatoes, slices were made through regions of infection and of noninfection. These were killed by submerging them in chromo-acetic acid for twelve hours. The slices were then washed under running water for twelve hours. After washing they were run through the standard method prescribed for imbedding tissues in paraffin.

Microtome sections were made of diseased tissue and stained with Fleming's triple stain. This proved to be unsatisfactory, however, so the sections were stained 5 minutes in safranin and 15 seconds in orange G with decidedly better results.

A strange fact is that Delafield's haematoxylin, so highly recommended for this type of tissue, proved quite unsatisfactory, even when sections were left in the stain for 4 days.

³ The colors mentioned in this discussion are named in accordance with "Color Standards and Nomenclature" 1912, by R. Ridgeway.

The use of Fleming's triple stain on microtome sections disclosed the fact that the hard portions of the tissue were made up of very compactly arranged host cells gorged with hyphae. The cell walls were frequently badly

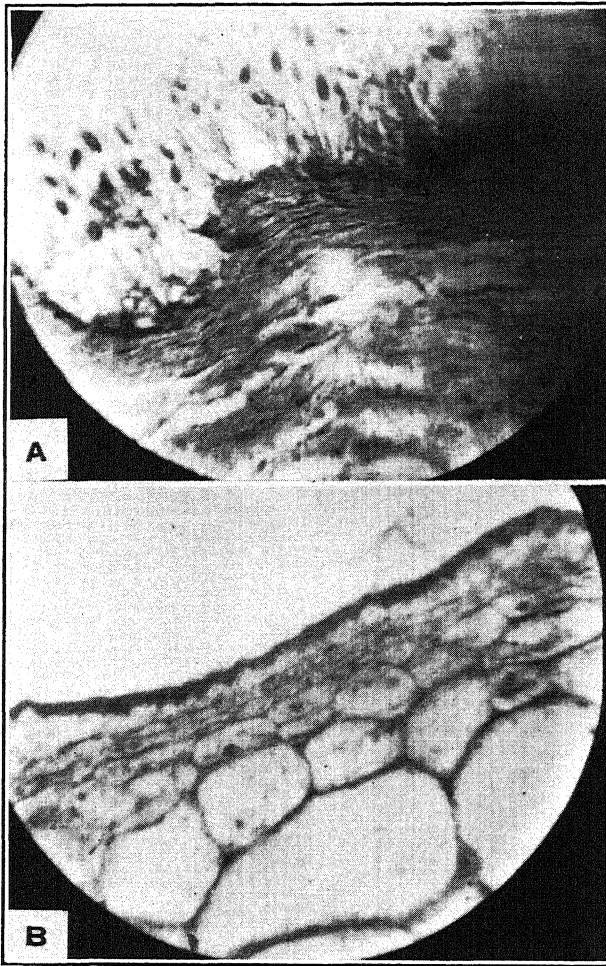


FIG. 3. Sections of tomato tissue. A. Diseased. B. Noninfected.

broken, and, where not broken, they had thickened considerably. The fungus frequently digests and absorbs the host tissue, walls as well as protoplasm, leaving large open spaces, thus causing the infected area to collapse and wrinkle in spots (Figs. 3 and 4). The transition from the infected area to the noninfected is a gradual one. In this respect the results differ from those of Pool (5), who found the transition to be very abrupt.

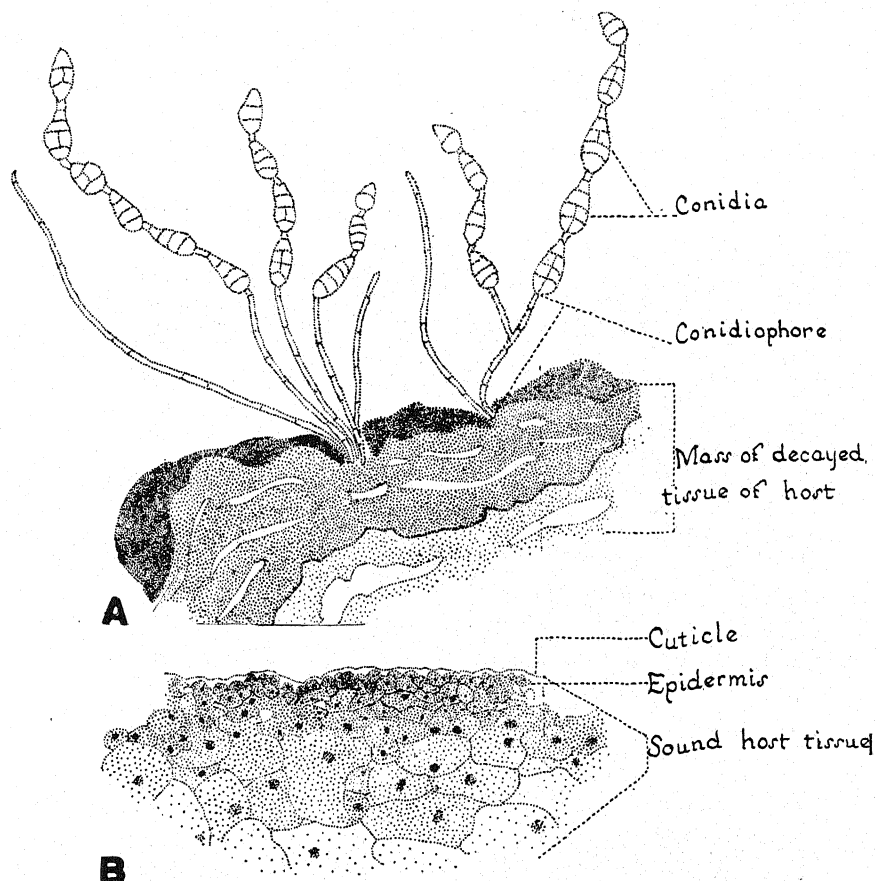


FIG. 4. Details from figure 3. A. Clusters of sterile and fertile conidiophores arising from disorganized and diseased host tissue. B. Noninfected host tissue. Both $\times 640$.

THE FUNGUS

Morphology

For the study of the fungus it was necessary to isolate it and grow it in pure culture. Potato agar, potato-dextrose agar, nutrient agar and tomato agar were used. Standard recipes were followed for all of the agars except the tomato. Growth on all but the tomato agar was unsatisfactory. On both potato and potato-dextrose agars the growth was black and formed a thin mat. On nutrient agar it was extremely slow, the mat being only 2 inches in diameter after 3 weeks. Growth on the tomato agar was a decided contrast, producing in from 3 to 5 days an average mat of 2 inches in diameter. After much experimentation it was found that the best agar was obtained when the following recipe was used: 30 grams of agar to 1000 cc. of

strained tomato juice. Although 30 grams seems an unusually large amount of agar for only 1000 cc. of juice, it was found that a less amount would not gel the liquid. The agars were all sterilized at 15 lb. pressure for 20 minutes.

Plate cultures made from portions of the diseased tissue resulted in pure cultures of the *Alternaria*. Inoculations made from these plate cultures on

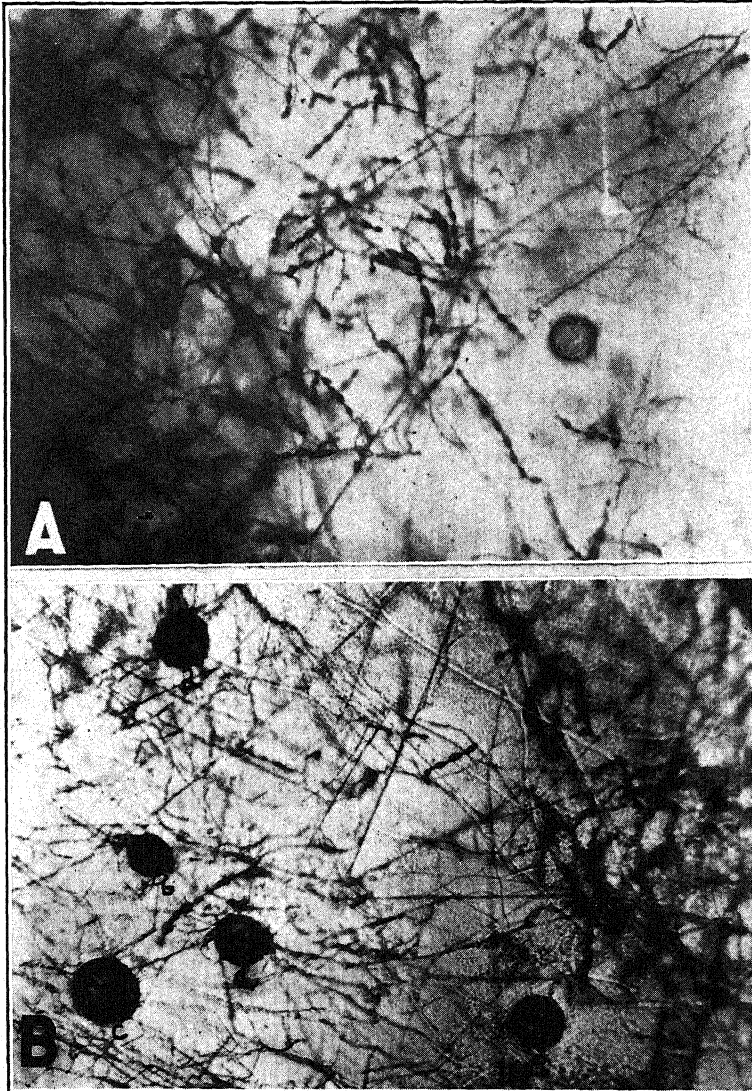


FIG. 5. A. Types of conidial chains. B. Large resting spores (a, b, c, d, e).

noninfected tomatoes reproduced the disease in every respect. Plate cultures made from these artificially infected fruits showed the organism to be the same as that previously isolated.

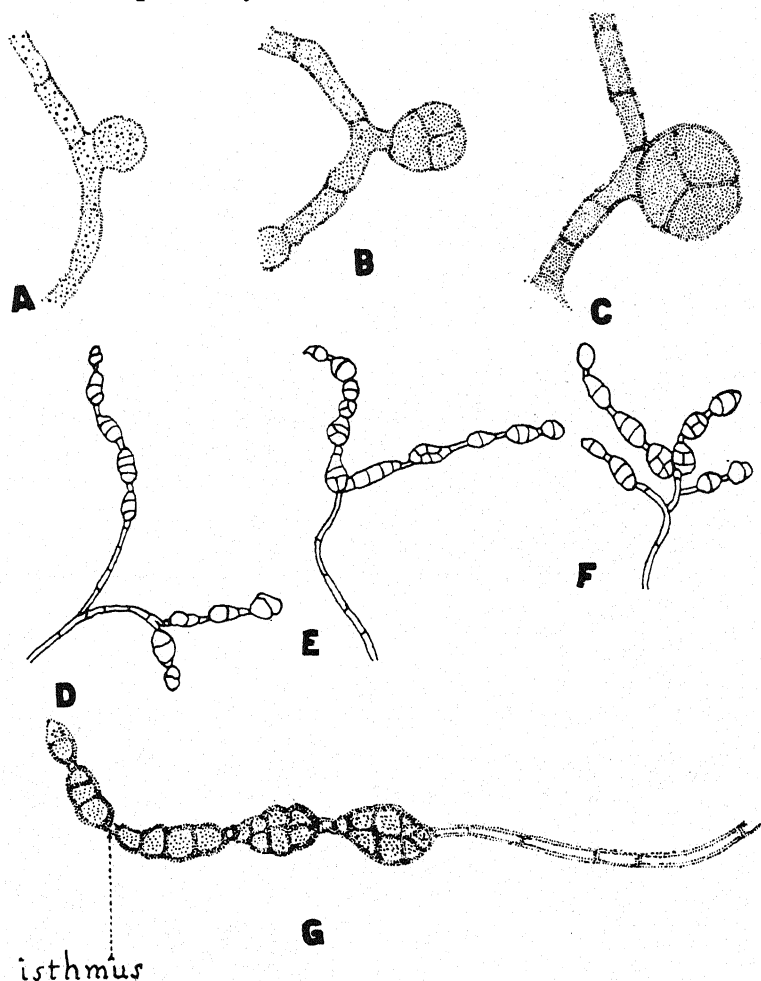


FIG. 6. A-C. Stages in the development of resting spores of *Alternaria* sp. D-G. Details of conidial branching from figure 5, A. A-C, G. $\times 500$. Others drawn under low power.

Microscopic examination of the fungus revealed that the fertile hyphae are at first hyaline to green, becoming tan to dark brown as the conidia form. Sterile filaments are always of a lighter color, frequently remaining hyaline throughout their life. Conidiophores, when mature, remain tea green and measure 59.2 to $74.0\ \mu$ by 1.6 to $2.2\ \mu$. Conidia are dark brown, measuring 20.8 to $36.0\ \mu$ by 3.2 to $9.6\ \mu$. These measurements differ from those of

Pool (5), which were much larger. Her conidia measured 35 to 66 μ by 16 to 20 μ , while her conidiophores measured 30 to 40 μ by 4 to 5 μ , and both her conidiophores and conidia were dark brown. The conidia in the experiment under consideration had 3 to 6 transverse and 1 to 3 longitudinal septa. The apical cell or isthmus connecting conidium to conidium was hyaline to dark brown, measuring 0.2 to 0.5 μ in length (Fig. 5, A), often producing a side branch that formed from 1 to several conidia (Fig. 5, A and D-F'). These side branches occurred frequently, being very characteristic. The conidial chains comprised conidia numbering 1 to 11 on a single branch. Pool (5) speaks of both her conidia and hyphae as being prominently echinulate, but the species here considered showed no tendency toward echinulation.

When the organism in the petri-dish cultures had almost completely digested the medium, large, round, four-cell spores 9.6 to 12.3 μ in diameter appeared. These were dark brown with very thick walls (Fig. 5, B, and Fig. 6, D to G). These spores never appeared unless the food became scarce. Time did not permit testing the fungus to determine whether the spores would develop as a result of merely lowering the temperature. But if these spores, which in later discussion are described as resting spores, were not present when a culture was placed in a freezing refrigerator, no matter how abundant the conidia, transfers from them showed no growth.

The author has not identified this species because none of the obtainable discussions of species already identified corresponded to the characteristics of the species under discussion. The *Alternaria* with which we are here concerned differed from published accounts of species in the following characteristics: (1) The number of conidia in a chain, which range from 5 to 11 as compared with 3 to 5 in species studied by others; (2) the isthmus is much shorter in comparison with that of other species; (3) the conidia are much smaller on an average and are less septate than those of many previously described species; (4) there is an abundant production of both conidia and resting spores on artificial media as well as on host tissue; (5) the fact that this species has two types of spores, while other species of *Alternaria* have only one. Whether this fungus is a new species or whether its apparent variations are merely reactions to environmental conditions, is a question that must be left to later investigation. It has been frequently observed that fungi, grown on different media or on different hosts, show considerable variation. The differences have been so pronounced that they have frequently been mistaken for distinguishing characteristics of new species. Therefore, before calling this *Alternaria* a new species rather than a habitat form, it would be necessary to grow it in varying environments and on different hosts.

However, one of the characteristics disclosed by this experiment is im-

portant enough to call for further discussion. *Alternaria* is not known to have differing types of spores, namely, conidia and resting spores. When this condition was first noticed it was thought that the culture was impure. To test this the following method of making plate cultures was followed. Shallow Petri dishes were poured with a layer of agar as thin as was possible and still cover the entire surface; with such a thin layer of agar the plate was quite transparent. Since dissecting needles become much roughened on the surface as the result of frequent flaming a new needle was used. It was sterilized in a 1-to-1000 solution of mercuric chloride. With this needle young cultures of *Alternaria* were barely touched and the needle thus infected was then used to streak the agar in the shallow plates above described. Examination of these plates by the low power microscope showed from 3 to 4 conidia in a streak, without any hyphae or other fungus material.

These plates were carefully checked from day to day and the spores were seen to germinate and grow. After extensive growth of the mycelium and abundant production of ordinary conidia, all of the 25 plates infected in this manner gave rise to round spores as soon as the agar was almost entirely digested. These plates were then frozen for 3 months, which is time enough to kill the conidia. Transfers made from the cultures produced the characteristic *Alternaria* conidia and again production of these resting spores when the food became scarce (Fig. 5, B).

PHYSIOLOGY

A number of agar-plate cultures were constantly maintained for experimentation. Tube cultures proved unsatisfactory for this fungus, because of the small surface of exposed medium. In order to observe closely the growth of the fungus from day to day, shallow Petri dishes were covered with a thin film of tomato agar and inoculated by either the streak or stab method. These Petri dishes were then placed in moist chambers and kept in a room-temperature incubator. Out of 800 plate cultures, pure cultures were obtained on all but 10 plates. These 10 were contaminated by *Rhizopus nigricans* or a *Penicillium*, introduced, no doubt, from another part of the laboratory where work was being done on these particular fungi.

To determine reaction to various environments, cultures were grown in light and dark places in the room where the temperature fluctuated between 15° and 24° C. Others were placed in the blood-heat incubator, room-temperature incubator, and in the refrigerator at 10° to 15° C.

Plates placed in the blood-heat incubator showed little or no growth. The mats increased only about 3.5 mm. in diameter before the culture died.

Cultures in the room-temperature incubator and those kept in the dark in the room where the temperature fluctuated from 15° to 24° C. grew satisfactorily. The type of growth of the cultures kept in the dark in the room

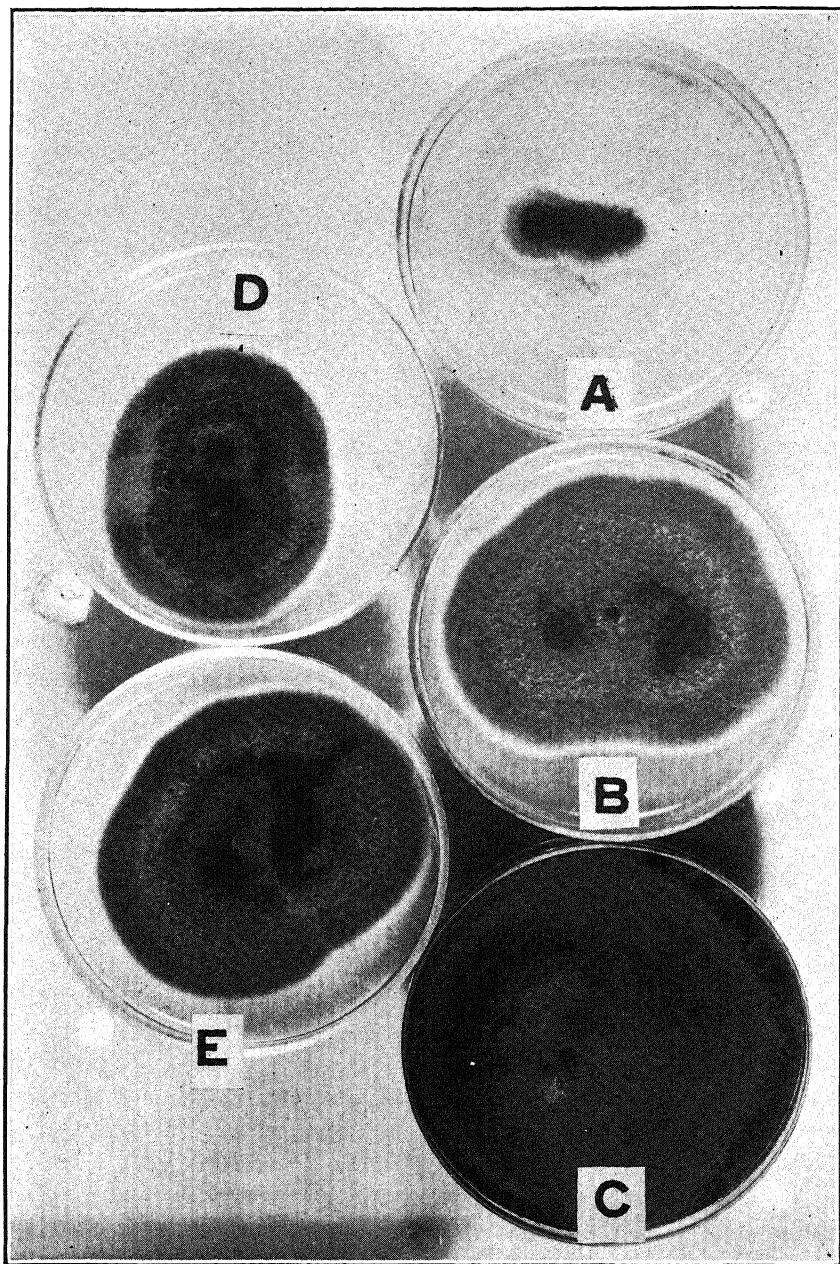


FIG. 7. Cultures of *Alternaria*. A. 6 days in incubator at room temperature. B. 2 weeks in incubator at room temperature. C. 6 weeks in room temperature (15°–24° C.) D. 10 days growth in room temperature (15°–24° C.) E. 2 weeks growth in room temperature (15°–24° C.).

was the same as that of those kept in the room-temperature incubator. The only difference was that the cultures grown in the room with fluctuating temperature showed a decided tendency to a variegated type of coloring. As long as the temperature remained low, growth was slight and a deep green. On the other hand, while the temperature was high, there was a pronounced increase in growth and a decided change in color from deep green to light tea green. The change from one color to another would be abrupt or very gradual, depending on the rapidity with which the temperature changed (Fig. 7, B-E).

The development of cultures placed in the light in the room was very similar to that of those grown in the dark, the only difference being in the size of the mat. Those grown in the light were about 2 cm. smaller in diameter, but the variegated coloring was still evident.

In the Petri dishes kept under the fluctuating room temperature the fungal mat of the *Alternaria* when 6 to 20 days old had a white margin, merging into tea green to dusky yellowish green towards the center. The very center was dusky olive green to black with gray to whitish wefts, either scattered over the entire surface or forming a complete border around it. The author was unable to determine the reason for this variegated appearance of the fungal mat when grown under conditions of variable room temperature. There was no apparent effect upon the hyphae except the change from deep green to tea green, according as the temperature was low or high.

Cultures kept in the refrigerator at 10° to 15° C. made little or no growth, and apparently none at all at 10° C. Some slight growth occurred at 15°; but in only 15 or 20 cultures did the mycelial mat increase. The growth was slow and unnatural in appearance, being pale green; and at the end of the 3 weeks during which they were kept in the refrigerator the average diameter of the culture was about 2 inches, or about 1/9 of what the growth would have been at room temperature.

An experiment was planned to determine the conditions in which the fungus overwinters. Cultures that had been grown in the room-temperature incubator for various lengths of time, ranging from 6 days to 6 weeks, were placed in the refrigerator and kept at -5° C. for periods of 7 to 90 days (Fig. 7). The results obtained are shown in table 1.

In experiments 4 and 5 (Table 1), in which growth was obtained on 27 of the 150 transfers, it is to be noted that these came from 9 of the 50 original plate cultures. These 9 cultures were grown in very shallow Petri dishes that held about 1/3 as much medium as other plates in the same experiment. In experiment 6 in which 6 out of 75 transfers failed to show growth, it should be noted that all 6 of these transfers were taken from 2 extra-deep original plates, holding about twice as much medium as those used in the remainder of the experiment. Consequently, all the agar was not entirely consumed.

TABLE 1.—*Results of transfers made from cultures of Alternaria sp. placed in the refrigerator*

Experiment No.	No. of plates used	Days under growth conditions at start of exper.	Days in refrigerator	No. of plates from which transfers were made	Total transfers made	No. of transfers showing growth	Percentage of transfers showing growth
1	25	6	7	25	75	0	0
		10	7	3	9	0	0
2	25	10	24	3	9	0	0
		10	90	19	57	0	0
		14	7	8	24	0	0
3	25	14	24	8	24	0	0
		14	90	9	27	0	0
		21	7	8	24	0	0
4	25	21	24	8	24	0	0
		21	90	9	27	3	11.1
		28	7	8	24	0	0
5	25	28	24	8	24	12	50
		28	90	9	27	12	44.4
		42	7	8	24	24	100
6	25	42	24	8	24	21	85.5
		42	90	9	27	24	88.8

Guba (3) reported that the viability of *Alternaria dianthi* extended over a period of only 2 winters. The evidence presented in table 1 shows that neither the mycelium nor the conidia of the species under discussion could survive even a week of freezing. This would indicate the necessity of a winter-hardy spore, capable of germinating on the restoration of favorable growing conditions. Such, apparently, is the function of the large, round resting spores, described in the foregoing. The absence of these spores explains the lack of growth in the 225 transfers made in experiments 1, 2 and 3, in that the young cultures from which the transfers were made, had produced no resting spores and the conidia were all killed by freezing. It explains also the large percentage of growth in the transfers made from the plates in experiments 5 and 6 in which enough time had been given for the exhaustion of the food supply and the consequent formation of resting spores. These spores were visible before the cultures were placed in the refrigerator. If the resting spores develop as the food becomes scarce, it would appear that, under natural conditions, they must be formed as the tomato fruits become more completely broken down and the available nutritive materials become exhausted by the fungus.

Plate cultures made from fruits frozen in the refrigerator showed no growth when the tomato used as the source of infection was only slightly diseased. However, when the plates were inoculated from thoroughly diseased fruits, 15 out of 25 showed growth. The explanation of the failure to produce growth when the disease is only slight lies in the fact that food is still abundant, no resting spores have been formed and none of the fungus is in condition to endure freezing. On the other hand, when the decay is considerable and the food supply in at least part of the fruit has given out, the resting spores are formed and freezing is not fatal to the fungus. Dissection of tissue from these thoroughly diseased fruits to determine the presence of resting spores was difficult because the tissue was extremely tough and not easily separable into thin sections. It was possible, however, to locate enough of the spores to verify their presence.

INOCULATION EXPERIMENTS

During the summer disease-free ripe fruits were removed from the vines and inoculated from Petri dish cultures by mechanical injury and insertion of mycelium. 5 to 6 days after inoculation these ripe fruits showed rotted areas measuring from $\frac{1}{2}$ to $\frac{3}{4}$ in. in diameter. In from 10 to 12 days the same fruits were rotted through $\frac{1}{3}$ to $\frac{2}{3}$ of their entire extent. Infected tissue thus produced resembled that produced by natural means in every respect, being hard, leathery, collapsed, and blackened.

Green fruits, also picked from the vines and inoculated in the same manner, required from 5 to 9 days longer than the ripe ones for the same amount of infection and decay to result, but they manifested the same characteristics, being hard, leathery, collapsed, and blackened. The only difference between the appearance of the disease on the green fruit and that on the ripe was that the ripe fruit was more wrinkled on the surface and the tissue was more collapsed.

Fruits inoculated in the same way, while still on the vine, responded in much the same way as did those inoculated after picking, the only difference being in the time necessary to bring about the same severity of infection. In the case of ripe fruit on the vine it required 8 to 10 days longer to develop the same amount of decay as obtained in ripe fruit inoculated after removal from the vine. In the case of green fruit on the vine, an infection equaling that of green fruit off the vine took from 10 to 15 days longer to develop.

Evidently, the decay is more rapid in ripe fruit than in green and more rapid in picked fruit than in non-picked (Fig. 8). Removal of the fruit from the vine lowers its resistance and makes possible a rapid penetration of the mycelium, probably because the characteristic cell turgidity is disturbed and the protoplasmic resistance is gradually overcome. The same would hold for ripe fruit as compared with green, except that, in the case

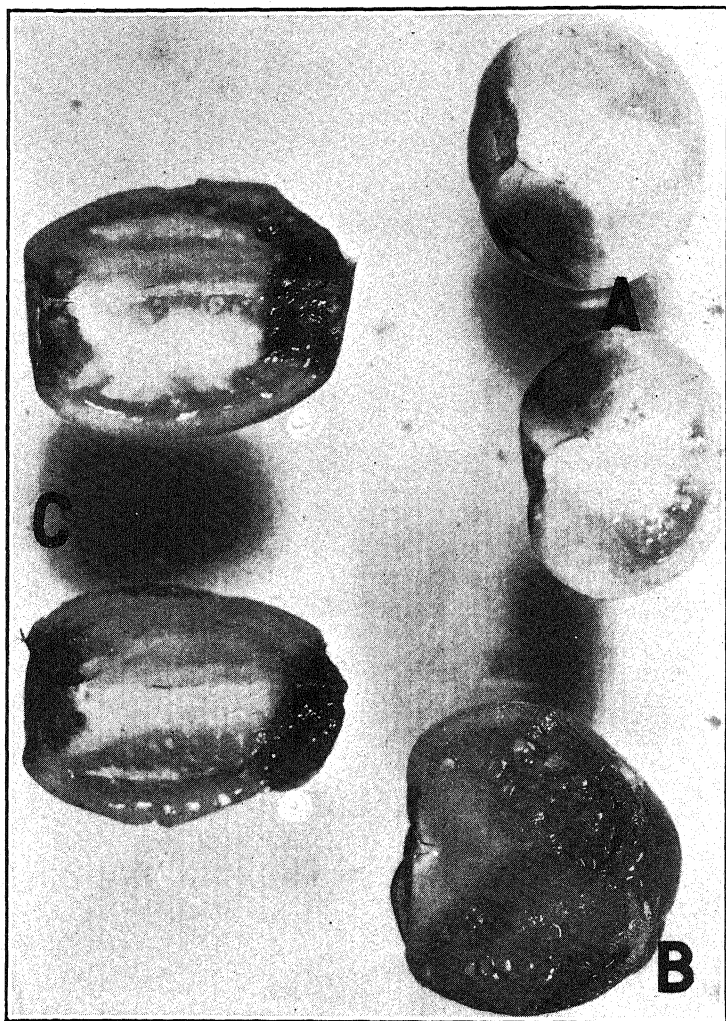


FIG. 8. Longitudinal sections through infected fruit inoculated after picking. A. Green fruit 12 days after inoculation. B. Ripe fruit 12 days after inoculation. C. Ripe fruit 5 days after inoculation.

of the ripe fruit, the cell walls have become more permeable, thus facilitating decay.

During the fall and winter, greenhouse experiments were performed to determine the method of infection under natural conditions. Seed was selected in the following manner. Two fruits were chosen, one of which was perfectly sound and the other decayed. They were placed in individual dishes to avoid contamination of the seed of the sound fruit by contact with

the infected fruit. The seeds of each were washed from the pulp. Since abortive seeds will float with the bulk of the pulp, sound seeds were easily obtained from among those that sank. A much larger proportion of the seed from the decayed fruit floated and was discarded. Seeds from the sound fruit, after being treated with a 1/10 per cent solution of mercuric chloride to reduce loss from various fungi, were planted in rich soil. The seed from the decayed tomato was not disinfected with mercuric chloride, since the spores of the infecting *Alternaria* might be carried on the seed.

The best 54 seeds from each source were selected for planting. One hundred per cent germination and growth occurred with the seed from the sound tomato, while only 22 per cent of that from the decayed tomato produced plants.

Microscopic examination of sectioned seeds showed that in some of the seeds the fungus had completely digested all the endosperm and almost all the ovule. In other seeds the infection was not so severe, for only a part of the endosperm and none of the ovule was absorbed. Probably the infected seeds that did produce plants were diseased only partially or not at all, while in those that germinated but were too weak to produce plants, the fungus had absorbed so much of the endosperm that there was not sufficient food left to support the seedling.

Eleven plants from seed of infected fruit and 11 from seed of non-infected fruit were planted in individual 8-in. pots of sandy loam. Soil in 6 pots of each of these 2 groups was inoculated by pouring on to it sterilized water to which had been added conidia of *Alternaria*. The remaining 5 plants in each group were reserved as controls.

One cluster of fruit on each of the 22 plants was bagged with cellophane during the entire period from the appearance of the blossom clusters until the ripening of the fruit. Another cluster on each plant was bagged only during blossoming; the bags were removed as soon as the fruit had set. When these bagged fruits were ripe they were carefully sectioned and in no case was the infection found. Evidently, neither infected seed nor infected soil is directly responsible for the black rot of the tomato.

In order to secure more normal growth the tomato plants were artificially lighted 14 hours daily. This not only resulted in stronger, stockier plants, but helped to shorten the time between seed planting and fruit production. The plants were carefully pruned to prevent excessive branching and were staked to facilitate more uniform exposure to light. Some branches were allowed to grow, but not enough to shut off light from the lower leaves.

Because of limited time the experiment in the greenhouse was made only with the variety Marglobe, chosen because of its short stigma, which would greatly facilitate self-pollination and therefore the setting of fruit in the absence of pollinating insects, and also because it had been most heavily infected in the open field during the preceding summer.

Fruits not bagged were contaminated by some one of the following procedures: painted stigmas, stab, surface contact or dust infection. In painting the stigmas sterile horsehair brushes were brushed over pure cultures of the *Alternaria* fungus. The brushes thus contaminated were then rubbed repeatedly over the ends of stigmas that were not over 3 days old. Most infection was received when the stigmas thus painted had been exposed to the air for only a few hours or for a day. Little or no infection occurred after the stigma was 3 days old.

Stab infection was accomplished by means of sterile needles, which, after being contaminated by scratching the pure culture of *Alternaria*, were used to prick or scratch the green or ripe fruit while still on the vine.

In preparation for dust infection an average-size frozen fruit, decayed by the fungus, was ground up with 300 grams of sterile soil. This mixture, after being thoroughly air-dried, was blown on newly opened flowers. The rest of the plant and other plants were carefully shielded from dust by means of newspaper caps.

Surface contact was secured by placing some of the fungus on the surface of the fruit without injuring the epidermis. Since the purpose of this second phase of the experiment was to duplicate in the greenhouse the conditions of the preceding excessively dry summer, to determine how the disease was spread, water suspensions of spores were not placed on the surface of leaves or fruit. This was because of the fact that in the garden during the preceding summer the moisture was so slight that for several successive weeks there was no dew. Douglas (1) found that infection occurred if the spores were suspended in water when placed on the surface of the fruit. He did not try the dry method of contact.

Leaves and stems of the tomato, inoculated by the same procedure as that followed in the inoculation of the fruit, gave negative results. Leaves and stems apparently are immune from the disease. Douglas (1) found that in some varieties these organs were susceptible to *alternaria* infection, while those of others were not. Marglobe may be resistant.

Results of the experiment with the growing fruit are shown in table 2.

Since none of the control clusters of fruit, whether from infected seed or not showed any spontaneous development of the disease whatever, it would appear that infection is not spread by infected seed. The fact also that these control clusters were not infected, even though many other clusters on each plant were heavily so, would indicate that the disease cannot pass from cluster to cluster through the stems. This opinion is strengthened by the fact that it was impossible to grow the fungus on the stems or leaves, regardless of the method of inoculation.

From table 2 it would appear that mechanical injury is most effective, since this method was 100 per cent efficient. But the possibility of any

TABLE 2.—*Results obtained from artificially infected fruits*

Method of treatment	No of fruits treated	No. of fruits infected	Percentage of fruits infected	No. of fruits not infected	Percentage of fruits not infected
Stab	55	55	100	0	0
Painting stigma ..	49	39	79.4	10	20.6
Blown dust	42	25	59.5	17	40.5
Surface contact	20	0	0	20	100
Control bagged	25	0	0	25	100
Control not bagged after formation of fruit	45	0	0	45	100

great amount of mechanical injury in the field, under natural conditions, especially when the vines are staked and the fruit lifted from the ground, would seem to be far less important than under the conditions of the experiment. In the field, infection probably occurs mostly by way of the stigmas. Table 2 shows that, of the total number of fruits subjected to blown dust inoculation, 59.5 per cent became diseased, while out of the total number contaminated by painting the stigma, 79.4 per cent developed the disease. This assumption is strengthened by the experience of Martin (4) who found that the disease was spread by insects and also could be carried on the pickers' garments.

The extreme danger of infection through mechanical injury when combined with the wind process is indicated in an incident that occurred during the course of the experiment. For a few weeks of the period during which the experiment was being carried on in the greenhouse, the place was infested by a sucking white fly that punctured the fruit, stems, and leaves of the plants in order to extract the juice. The plants around a certain cluster of young flowers had been covered with newspapers in preparation for blowing the dust on the young stigmas. The ventilators of the greenhouse were open and just as the dust was being blown a gust of wind came in and spread the papers apart in line with the inoculation dust, thus exposing a cluster of fruit that had been punctured by white flies. On this cluster around each fly puncture there was alternaria infection. Although this is an isolated instance, it does seem to strengthen the evidence for wind inoculation. It should be noted that Gibson (2) reported aphid injury as contributing to the spread of alternaria infection in soy beans and cow peas. How effective wind inoculation can be is evident when compared

with the results obtained by the stab method as shown in table 2 in which 100 per cent of the fruits treated by the latter method shows infection. In other words, wind inoculation would be effective whether the spores were blown on new stigmas or on wounded fruit. Apparently, infection occurs more readily through injury than through the stigma, since 29.7 per cent of the stigmas treated did not take the infection, while mechanically injured fruit was 100 per cent susceptible. Under natural conditions, however, there is always an abundance of young stigmas subject to inoculation, while mechanical injuries are rare in comparison.

Infection of some fruit by surface contact would seem to be unimportant in the absence of accumulated moisture. Table 2 shows no infection by this method. Apparently, the fungus is unable to grow on the surface of the tomato and penetrate the stomata. This is further confirmed by the fact that, in the accident above referred to, infection from the spore-laden dust occurred only where the surface of the fruit had been punctured by sucking insects. This does not, of course, invalidate the results obtained by Douglas (1), who employed the method of surface contact by means of spores suspended in water. Where fruit of unstaked plants is allowed to lie on damp, infected ground, direct infection from resting spores or conidia might well be the main source of black rot.

The results obtained by employing these methods of inoculation, stab, painted stigma, wind, and surface contact, seem to suggest that the method of infection is primarily stigmatic, with secondary infection from mechanical injury by insects or otherwise, followed by infection-laden winds or by the actual spread of the disease by the insects as they crawl over the mechanical injuries from infected to uninfected regions.

CONCLUSION AND SUMMARY

In conclusion, it would appear that the cause of black rot in tomato is a species of *Alternaria* whose hyphae enter the fruit either through the stigma of a recently opened flower or through fresh wounds made by some mechanical injury, as, for example, the bite of an insect. It would appear that the infection is spread principally by winds; but the results do not preclude the transportation of the spores on the bodies and legs of insects, passing from infected to noninfected fruits.

Infection seems to occur in the fruit only, resulting in a dark brown, leathery spot, frequently occupying one-half of the tomato. This is the spot on which the conidia are produced from rapid spread of the disease. When the fruit is badly decayed so that the food becomes scarce, large resting spores are produced. Fruit, dropping to the ground, decays leaving the conidia and resting spores in the soil. The winter, if severe, kills the conidia, while the resting spores live on to be carried by the wind-blown

dust of the following season to reinfect the flowers of the new crop. In the first infected fruits conidia are again produced and are blown or carried by insects to further spread the disease.

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FUNGI OF SUGAR BEETS¹

F. ALLEN HODGES

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INTRODUCTION

Various phases of the sugar-beet industry bring about situations in which beets are stored or piled temporarily. In seed production, beets must be stored through the winter. Including then, beets grown from seed, seedlings, maturing beets in the field, and the subsequent storing of beets, whether it be temporarily on the way to the sugar factory for slicing, or for longer periods, such as through the winter, to seed production; herein are involved many phases in which the fungal flora of beets may be of importance.

This paper comprises the study of the complete fungal flora of some 5000 beets grown at Syracuse, N. Y., and other places throughout the country.

All of the fungi found present on or in beets in the field and in storage were cultured. These include every form of fungus whether or not the form may hitherto have been considered as of economic importance. Such common forms as *Penicillium*, *Cephalothecium*, *Rhizopus*, and others have been given consideration.

HISTORICAL

A number of fungi already have been reported as occurring on beets, but no comprehensive list has been compiled. Duggar (2), in 1899, published his "Three important fungous diseases of the sugar beet," and Edson (4), in 1915, published a more limited work on "Seedling Diseases of the Sugar Beet and their Relation to Root-Rot and Crown-Rot." These two publications give only a meager survey of the fungi occurring on beets. Numerous forms of fungi attacking beets have been reported from time to time from this country, Europe, and Asia. Nakata *et al.* (8) have studied the diseases of beets in Korea. Seymour (10), in his "Host Index", lists various forms from beets in this country. Most of the work has been reported from Europe where the sugar-beet industry has been well-established for many years.

SOURCES OF FUNGI

The fungi recorded in this paper were secured from beets in storage, in the field, and from those grown in the greenhouse; also, from seedlings grown in the field and laboratory.

¹ Thesis, in part, submitted to the Graduate School of Syracuse University in partial fulfillment for the degree of Doctor of Philosophy.

The writer wishes to offer grateful acknowledgement to Dr. Ernest Reed, of Syracuse University, who directed this research and gave generously of his assistance and advice throughout the investigation.

A species of the genus *Fusarium* was of greatest frequency and was most common in stored beets, though it was secured also from field-grown seedlings, from beet seed, and from beets that had remained in the field over winter.

Alternaria occurred on all parts of the foliage of beets. It was obtained from lesions on the petioles and from leaf spots on the blade, and was found also on beets growing in the greenhouse and in the field, there producing various types of leaf spot.

Species of *Rhizoctonia* and *Fusarium* were isolated from seedlings grown in the greenhouse. *Rhizoctonia* was by far the most destructive of the two on the seedlings.

Phoma betae and *Sphaeropsis* sp. also were responsible for storage rots, but only to a slight degree. *P. betae* also was secured from seedlings grown in sterilized soil in the laboratory and killed by the fungus.

A few saprophytic fungi were found on beets in storage. Among these were 4 species of *Penicillium*, *Cephalothecium roseum*, *Acrostalagmus cinabarinus*, and a species of *Arthrinia*. All of these were on the surface of the beets. *Rhizopus* was not noticed at first as a very destructive fungus in storage, but later was found in the inoculation experiments to be an important cause of decay.

Many species of *Fusarium* were obtained from sugar beets from various

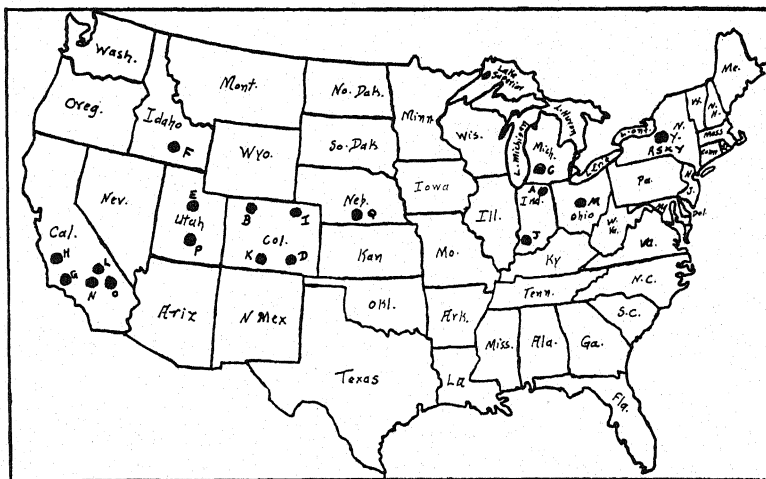


FIG. 1. Localities from which species of *Fusarium* were obtained from beets: A. Decatur, Ind.; B. Loveland, Colo.; C. St. Louis, Mich.; D. Eaton, Colo.; E. Salt Lake City, Utah; F. Preston, Idaho; G. Betteravia, Calif.; H. Santa Ana, Calif.; I. Rocky Ford, Colo.; J. Vincennes, Ind.; K. Sterling, Colo.; L. Manteca, Calif.; M. Fremont, Ohio; N. Oxnard, Calif.; O. Spreckles, Calif.; P. Ogden, Utah; Q. Bayard, Neb.; R, S, X, and Y. Syracuse, N. Y.

sections of the United States. The States from which these forms were collected are shown on the accompanying map (fig. 1).

METHODS

Since the purpose of this work was to undertake as comprehensive a study as possible of all the forms of fungi found in connection with beet, much detailed study was given to the isolation and culture of these fungi and to their description. A considerable part of the paper, therefore, is of a taxonomic nature, dealing with a description of each form listed as follows:

Phoma betae (Fr.), *Rhizoctonia* sp., *Penicillium roseum* (Link), *P. digitatum* (Fr.) Sacc., *P. commune* Thom., *Acrostalagmus cinnabarinus* (Cda.), *Cephalothecium roseum* (Cda.), *Alternaria* sp., *Sphaeropsis* sp., *Arthrinia* sp., *Mucor heterosporus* (Fisher), *Rhizopus nigricans* (Ehr.), *Cylindrocarpon radicolica* (Wr.) var. *violaceum* Hochapfel ad int., *C. didymum* (Hart.) Wr., *C. radicolica* Wr., *Fusarium arcuatum* Berk. et Curt., *F. arcuoporum* Sherb., *F. bulbigenum* Cke. et Mass., *F. bullatum* var. *minus* Sherb., *F. chenopodium* (Thuem) Sacc., *F. clavatum* Sherb., *F. culmorum* (W. G. Sm.) Sacc., *F. dimerum* Penz., *F. dimerum* Penz. var. *pusillum* Wr., *F. discolor* App. et Wr., *F. diversisporum* Sherb., *F. equiseti* (Cda.) Sacc. var. *bulbatum* (Sherb.) Wr., *F. equiseti* (Cda.) Sacc. var. *crassum* Wr., *F. ferruginosum* Sherb., *F. herbarum* (Cda.) Fries, *F. heterosporum* Nees, *F. lateritium* Nees, *F. lutulatum* Sherb., *F. martii* App. et Wr., *F. merismoides* Cda., *F. merismoides* Cda. var. *majus* Wr., *F. moniliforme* Sheldon, *F. orthoceras* App. et Wr., *F. orthoceras* App. et Wr. var. *albido-violaceum* (Daz.) Wr., *F. orthoceras* App. et Wr. var. *longius* (Sherb.) Wr., *F. orthoceras* App. et Wr. var. *triseptatum* Wr., *F. oxysporum* Schl., *F. radicolica* Wr., *F. redolens* Wr., *F. sambucinum* Fekl., *F. scirpi* Lamb. et Fautr., *F. scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr., *F. scirpi* Lamb. et Fautr. var. *caudatum* Wr., *F. scirpi* Lamb. et Fautr. var. *compactum* Wr., *F. scirpi* Lamb. et Fautr. var. *filiferum* (Preuss) Wr., *F. semitectum* Berk. et Rav., *F. semitectum* Berk. et Rav. var. *majus* Wr., *F. solani* (Mart.) App. et Wr., *F. solani* (Mart.) App. et Wr. var. *martii* (App. et Wr.) Wr., *F. solani* (Mart.) App. et Wr. var. *medium* Wr., *F. solani* (Mart.) App. et Wr. var. *minus* Wr., *F. solani* (Mart.) App. et Wr. var. *suffusum* Sherb., *F. sporotrichioides* Sherb., *F. subulatum* App. et Wr., *F. trichothecioides* Wr., *F. truncatum* Sherb., *F. ventricosum* App. et Wr., *F. viride* (Lechm.) Wr., *F. flocciferum* Cda., *F. reticulatum* Mont.

In the cultural studies several types of media were used. It was thought that the different media would provide possibilities for fungus variations that might occur in response to the nutriment furnished. No variations,

however, were observed and any one of the several media would have served the purposes fully as well as any other. The media used were:

1. Synthetic (maltose 6 grms. per liter) (6), 2. Edson (dextrose 100 grms. per liter) (4), 3. Cornmeal (3), 4. Red beet (3), 5. White beet (3), 6. Rice.

The identification of the species and varieties of *Fusarium* was determined chiefly through the work of Wollenweber's (13) "*Fusaria Autographice Delineata*." Other sources were consulted and include the following:

Appel and Wollenweber (1), Wollenweber (12-14), Sherbakoff (11), Reinking and Wollenweber (9), Wollenweber *et al.* (15), Link and Bailey (7).

A number of the cultures were sent to Dr. Wollenweber for identification.

Inoculation studies were made in which the matured beet under storage conditions was used, and the effect of inoculations on growing beets in the seedling stage was observed.

BEETS IN STORAGE

Three sets of cultures were set up. Two of these consisted of the inoculation of beet roots, placing them in boxes of moist sand, and storing them in the laboratory at room temperature (21° C.).

For these experiments the beets first were washed in water to remove the dirt. They were then washed in a 1:1000 solution of mercuric chloride and allowed to dry. In order to introduce the fungus into the beet, a hole was made completely through the latter near the crown by means of a 5 mm. cork borer. Another device used to make a hole through the beet was a $\frac{1}{4}$ inch bit attached to an electric motor.

Stock cultures of the fungi served as inoculum. Pieces of the mycelium, masses of spores, sporodochia, or piconotes, as the case might be were placed in the hole of the beet, and the ends of the hole were plugged with sterilized cotton. The beets were labeled and packed in moist sand. Figure 2, C, represents a noninoculated beet, used as a control.

In the first set of experiments 3 beets were inoculated with each fungus. The beets thus inoculated were labeled and packed in boxes of sand. At the end of a period of about 4 weeks, the beets were removed and examined. In some instances it was observed that the fungi produced excellent symptoms of rot, while in others no penetration took place. Other beets were so rotted by fungi that were not used as inoculum that results were unsatisfactory. There also was loss of material due to destruction of the labels.

The second set of experiments was conducted similarly, but only those fungi were used that gave unsatisfactory and inconclusive results in the

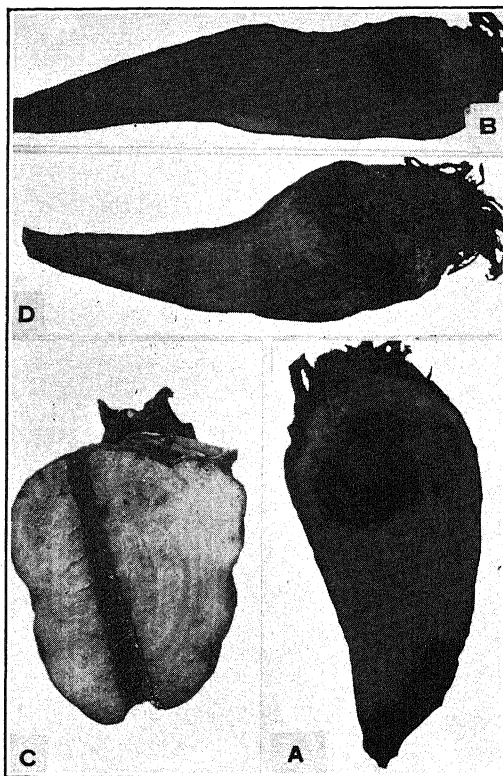


FIG. 2. A. Inoculated beet showing the rot produced by *Fusarium orthoceras*. B. Beet inoculated with the fungus *F. dimerum*. C. Sugar beet used as a control, shows the hole through the beet for inoculation purposes. D. Inoculated beet, showing the rot produced by the fungus *F. ventricosum*.

first set. For this experiment 24 beets were inoculated with 8 fungi and placed in a single box of sand. By employing this method the boxes were not overcrowded and the beets could be more evenly spaced. They were prepared and inoculated in the same manner as in the preceding experiment.

The boxes were stored in the laboratory, but the beets were not left in the sand so long as were those of the first set of experiments. At the end of 2 weeks, the beets were removed and examined. It was found, however, that the results were even less satisfactory. There was considerable loss of material due to contamination by bacteria and other fungi. However, some of the beets were unaffected by contaminations and showed good results from the inoculations.

Although the results obtained in these two experiments were not fully satisfactory, yet the amount of damage done to the beets that were rotted by some of the fungi, indicated the importance of the latter as rot-producing

organisms of stored beets. Those forms that were considered most active in producing rots and, therefore, the most destructive, are listed below.

Phoma betae, *Rhizoctonia* sp., *Sphaeropsis* sp., *Fusarium dimerum*, *F. orthoceras*, *F. semitectum* v. *majus*, *F. ventricosum*, *F. diversisporum*, *F. arcuospurum*, *F. merismoides*, *F. solani*, *F. chenopodium*, *F. clavatum*, *F. bullatum* v. *minus*, *F. trichothecioides*, *F. radicola*, *F. scirpi* v. *filiferum*.

Some of the rots produced in these stored beets by a few of the species of *Fusarium* listed above are shown in figure 2, A, B, and D.

The third set of experiments consisted in placing the inoculated beets in damp chambers instead of storing them in boxes of sand. This method was accomplished by putting a single inoculated beet into a quart Mason jar. One beet was used for each fungus. All of the jars were sterilized in the autoclave and tap water used; the water changed every day during the course of the experiment, with just enough to cover the bottom of the jar.

The beets were washed, sterilized, and inoculated as in the preceding experiments. The jars were covered with a piece of cotton and the jar tops inverted on them.

The sets of inoculations were run for a period of 7 days. The beets were then removed and the amount of penetration by each fungus was determined by carefully cutting through the beet at the place of inoculation.

The fungi varied in their ability to penetrate the beet tissue. Some were rapid growers and produced excellent penetration, even to enlarging the hole through the beet. Others showed no sign of penetration in contrast with the cultures of the first experiments. In cases where contaminating fungi were found attacking the surface of the beet, the infected part was cut off to check any further development of the lesions. Many of the beets induced the growth of thick, matted roots and leaves.

This method of inoculation seemed the most satisfactory in that it showed whether the fungus was capable of penetrating the tissues of the growing host, and of entering the turgid cells. It, therefore, afforded a better idea as to the real pathogenicity of the fungi than did the other experiments, and it also offered less opportunity to contaminating forms.

In addition to fungi listed above, the following species of *Penicillium*, *Rhizopus* and *Fusarium* may be considered as important causes of storage rots of the beet: *Penicillium commune*, *Rhizopus nigricans*, *Fusarium culmorum*, *F. arcuatum*, *F. discolor*, *F. dimerum* v. *pusillum*.

As in the other experiments, the fungus *Fusarium dimerum* was found to produce the most damage to beets in the damp chamber.

The results obtained from these experiments involving inoculation of beet roots would indicate that some of the fungi are much more destructive than others, while some are quite innocuous. Especially in the case of the damp-chamber cultures, where the beets were growing to a slight degree,

penetration by the fungus was more significant in that a good penetration would indicate a strong pathogenic nature of the fungus. Where no, or only a slight, penetration was obtained, the fungus would be of lesser importance and cause only a slight loss of beets in storage.

Fusarium dimerum and *F. orthoceras* appeared to be most actively parasitic in that they produced the most extensive penetration in the roots in all of the experiments.

Of the 85 species and varieties of fungi used in these experiments, only 23 showed any appreciable effect on stored beets and some of these produced only slight penetrations.

SEEDLING INOCULATION EXPERIMENTS

Another phase of the experimental study of the fungi of beets, was that made to find out what effect, if any, such fungi would produce on the growing plant. To this end seedlings were grown in the laboratory and in the field.

Three-inch flower pots of soil were sterilized in the autoclave at 15 pounds pressure for 1 hour on 2 successive days. Seeds of the variety Menomonee were planted in the pots, and before planting, the seeds were rinsed in a 1:1000 solution of mercuric chloride, followed by rinsing in distilled water. From 10 to 15 seeds were planted in each crock and the crocks kept in the laboratory at room temperature, covered with sterilized Petri dish covers.

As soon as the seedlings showed above the surface of the soil, spore suspensions, in boiled tap water, were made from each fungus. These suspensions were made by filling a test tube (about 25 cc. capacity) with the boiled tap water and putting into it masses of mycelial tissue. The tube was then shaken thoroughly. One tube of these spore suspensions was made for each fungus used and it was poured over and around the seedlings in a crock. One crock was used for each fungus. At the end of about a week the seedlings were examined to note any effect the fungi may have had upon them. Those seedlings that showed any injury were carefully removed from the flower pots, washed in distilled water to remove the dirt, dipped in mercuric chloride (1:1000), rinsed in distilled water, and placed in Petri dishes containing the synthetic medium (6). On development of the fungus in culture, it was identified and its characteristics were studied.

The results of this study showed that only about 20 per cent of the fungi employed produced positive results and were capable of causing seedling infection. From this it would appear that the fungi have, in most cases, little, if any, effect on beet seedlings and need not be considered as important pathogens of the growing beet.

As was found in the previous inoculation experiments, *Fusarium*

dimerum and *F. orthoceras* proved to be the most active parasitic forms. They produced from 50 to 75 per cent infection in the seedlings.

Inoculation studies of seedlings grown in the field were made chiefly with the numerous forms of the genus *Fusarium* obtained from various parts of the country. (Fig. 1). Details of the results obtained will be considered later. Suffice it to be stated here that, in general, the results were negative and only a few species attacked the seedlings. Of these, *Fusarium orthoceras* was as active a parasite under field conditions as on beets in storage.

TYPES OF ROTS PRODUCED BY THE FUNGI IN STORED BEETS

During the course of the investigations on inoculation of beets in storage, it was noticed that the fungi used produced different types of rot.

Three such rots were noted: (1) A distinctly dry, corky rot, producing in some cases enlargement of the hole through the beet. This enlargement

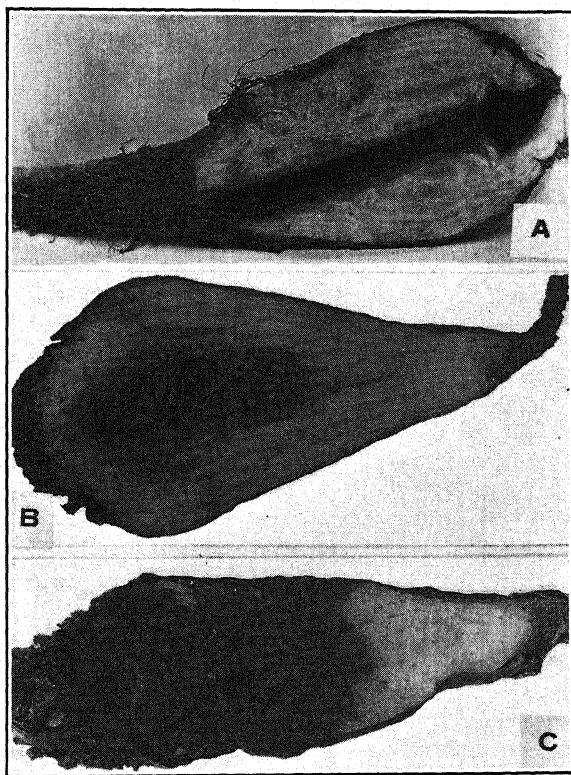


FIG. 3. A. Dry, corky rot produced by *Phoma betae*. B. Soft, watery rot also produced by *P. betae*. C. Intermediate type of rot produced by the fungus *Fusarium orthoceras*.

was of different degrees in the various cases noted. Fig. 3, A, shows such a rot produced by *Phoma betae*. (2) A soft, slimy type that produced a wet, water-soaked appearance in the beet. Fig. 3, B, shows this type and it also was produced by the fungus *Phoma betae*. (3) A third type was one that was neither a corky nor a wet rot. The fungus penetrated the substratum, destroyed the tissue, produced a type of rot that could not be designated either as a dry, corky rot nor yet as a wet rot. It seemed to be a type in which many cavities were formed, and in some cases these cavities were filled with a mycelial growth. This was designated as an intermediate rot. Fig. 3, C, shows this type produced by *Fusarium orthoceras*.

These observations were recorded at the time the beets were examined to study the effects produced by the inoculations with the various fungi. This represented merely additional data to the inoculation studies and no further detailed study was given these observations.

GENERAL DISCUSSION

The material used in this investigation was secured from sugar beets grown at Syracuse, N. Y., and in various other parts of the United States. The distribution of the localities which supplied material is shown in figure 1. The fungi used in this study have been secured from beets grown in the field, from beets in storage, and from beet seedlings grown in the field, laboratory, and greenhouse. Beet seed obtained from Europe was the source of some of the fungi studied.

Only a few forms of those fungi previously mentioned in the literature were found during the progress of these studies. These are as follows:

Phoma betae, *Rhizoctonia* sp., *Alternaria* sp., *Rhizopus nigricans*, *Pionnotes betae*, *Fusicola betae*, *Cephalothecium roseum*, *Fusarium argillaceum*, *F. reticulatum*, *F. ventricosum*, *F. merismoides* v. *majus*.

The following forms: *Fusarium argillaceum*, *Fusicola betae*, and *Pionnotes betae*, mentioned by others, may all now be considered as species of the genus *Fusarium*. Wollenweber (14) reports only a few *Fusarium* species found on beets. He states that the name *F. betae*, which is synonymous to Saccardo's *Pionnotes betae*, is now changed to *Fusarium merismoides* v. *majus*. In his *Fusaria Autographice Delineata* (13) he states that *F. betae* is a synonym for *Cercospora beticola* (Sacc.), which is reported as attacking the foliage of beets. *Fusisporum argillaceum* (Fries.) he identifies as *Fusarium argillaceum* (Fr.) Sacc. and to which he gives the synonymous name *Fusarium ventricosum* App. et Wr.

The following fungi comprise those here reported for the first time as prevalent on the beet:

Penicillium roseum, *P. digitatum*, *P. commune*, *Acrostalagmus cinna-*

barinus, *Sphaeropsis* sp., *Arthrinia* sp., *Mucor heterosporus*, *Cephalosporium* sp., *Spicaria griseola*, *Haplosporella* sp., *Cylindrocarpon radicicola*, *C. radicicola* v. *violaceum*, *C. didymum*, and *Fusarium* sp. (about 49 spp.) and var. as listed on page 561.

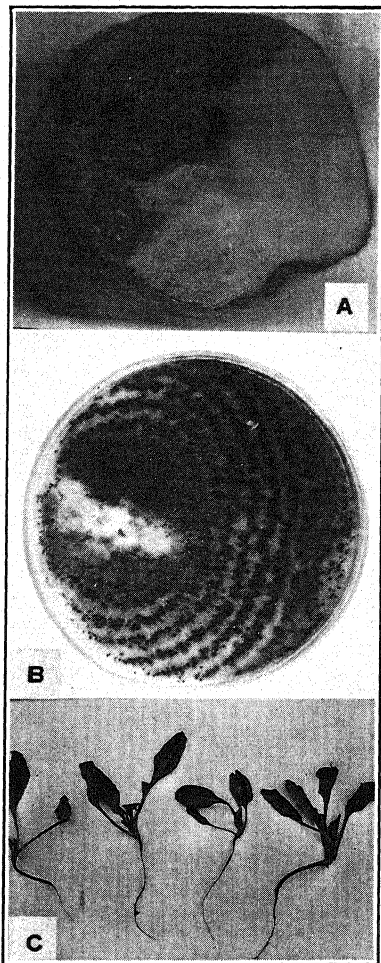


FIG. 4. A. Rot produced by *Phoma betae* in a stored sugar beet. B. Culture of *P. betae* on corn-meal medium, showing the production of pycnidia. C. Root-rot of sugar beet seedlings produced by the fungus *Rhizoctonia* sp.

Though *Phoma betae* often has been reported as a destructive fungus of beets and has been mentioned recently by Grooshevoy (5) as the cause of a serious root rot of beet seedlings, it was not found to be so prevalent in this investigation (Fig. 4).

Cercospora beticola causes widespread defoliation of beets in certain sections of this country and Europe, yet it was not abundant under observation in this study. This may be due to the fact that the summers of this locality are cooler than in those areas where *Cercospora* is so prevalent.

Alternaria sp. was found as a leaf spot in this locality. The damage done by *Alternaria* does not seem to be so vital as that caused by *Cercospora*, since it does not defoliate beets, whereas *Cercospora* has been known to do so, 2 and even 3 times in a season. Various types of leaf-spot infection and petiole lesion were found attributable to this species of *Alternaria*, hence it was regarded as a parasite and was found to cause a slight defoliation of beets growing in the field.

Beet rust, beet mosaic, and bacterial diseases of beets were not observed in this study. The beet nematode, *Heterodera schachtii*, presents a real problem for the beet growers in some of our western States, but not in this region.

Though *Rhizoctonia* sp. produced some decay in stored beets, it was more destructive of seedlings. (Fig. 4, C.)

The genus *Fusarium* has been considered of no very great importance in beet culture; but, in the light of these studies, it appears that *Fusaria* probably cause the largest number of rots in beets. The number of species of *Fusarium* secured from beets was large. These have been studied on the assumption that they may all differ in some respects. The data have justified this assumption, for it will be noted that certain *Fusaria* that appear taxonomically identical are not alike in other respects. Many duplicate cultures were, therefore, maintained and each treated as a separate and distinct species. The various species collected and studied have been shown to be more important as storage-rot fungi, and only a very few proved to be parasitic on the beet seedlings. There were certain forms, however, that had little or no effect on stored beets.

In the studies made on the inoculation of beets in storage, it was observed that different types of rot were produced by the fungi used. The results indicated that a few of the forms were capable of producing all types of rot, namely; a dry, corky rot; a wet, slimy rot; and what was termed an intermediate rot. Other forms produced only one type. This phase of the work, however, was merely suggestive and is presented in the form of additional data. Conclusions are withheld pending further observations.

Referring again to those fungi considered in this paper and listed on page 561 the question arises as to which of these forms are parasitic and which are merely secondary invaders. Those forms attacking the growing beet and those causing considerable decay of beets in storage are considered as parasitic fungi. The secondary invaders may be included in

TABLE 1.—Fungi isolated from beets and arranged according to their economic importance

Group A. Parasitic fungi			Group B. Saprophytic fungi (stored beets)	
Beets in storage	Seedlings in field	Seedlings in laboratory	Cause little or no decay	Of no economic importance
<i>Phoma betae</i>	<i>Fusarium</i>	<i>Rhizoctonia</i> sp.	<i>Cylindrocarpon radicola</i>	<i>Penicillium</i>
<i>Rhizoctonia</i> sp.	<i>orthoceras</i>	<i>Penicillium roseum</i>	var. <i>radicola</i>	<i>funiculosum</i>
<i>Sphaeropsis</i> sp.	<i>Fusarium</i>	" <i>digitatum</i>	" <i>radicola</i>	<i>Acrostalagmus</i>
<i>Penicillium commune</i>	<i>solani</i>	<i>Rhizopus nigricans</i>	<i>Fusarium arcuosporum</i>	<i>cinnabarinus</i>
<i>Rhizopus nigricans</i>	<i>Fusarium</i>	" <i>orthoceras</i>	" <i>bulbigenum</i>	<i>Mucor heterosporus</i>
<i>Fusarium arcuatum</i>	<i>merismoides</i>	" <i>equiseti</i>	" <i>ferruginosum</i>	<i>Cylindrocarpon</i>
" <i>bullatum</i>		" <i>v. bullatum</i>	" <i>herbarum</i>	<i>didymum</i>
" <i>v. minus</i>		" <i>dimerum</i>	" <i>heterosporum</i>	<i>Cephalothecium</i>
" <i>chenopodium</i>		" <i>v. fliferum</i>	" <i>lateritium</i>	<i>roseum</i>
" <i>clavatum</i>		" <i>solani</i>	" <i>merismoides v. majus</i>	
" <i>culmorum</i>		" <i>lutidum</i>	" <i>moniliforme</i>	
" <i>dimerum</i>			" <i>orthoceras v. longius</i>	
" <i>dimerum</i>			" <i>orthoceras</i>	
" <i>v. pusillum</i>			" <i>v. triseptatum</i>	
" <i>discolor</i>			" <i>oryzporum</i>	
" <i>diversisporum</i>			" <i>redolens</i>	
" <i>merismoides</i>			" <i>sambucinum</i>	
" <i>orthoceras</i>			" <i>scirpi</i>	
" <i>radicola</i>			" <i>v. caudatum</i>	
" <i>semitectum</i>			" <i>v. acuminatum</i>	
" <i>v. majus</i>			" <i>sclerotioides</i>	
" <i>trichothecioides</i>			" <i>semitectum</i>	
" <i>ventricosum</i>			" <i>solani v. martii</i>	
			" <i>v. suffusum</i>	
			" <i>sporotrichioides</i>	
			" <i>subulatum</i>	
			" <i>truncatum</i>	
			Several unidentified <i>Fusaria</i> spp.	

the list of the saprophytic fungi, which, while they occur on beets in storage, may not be decay producing organisms. From this point of view, therefore, an attempt has been made to provide a list of those fungi that might be considered of economic importance to the sugar beet industry and those that are of little or no importance. Table 1 presents a summary of these forms from this economic point of view. Those forms that fall under group A—parasitic fungi—may be considered as those that would be most destructive to beets both in storage and as seedlings; while those of group B—the saprophytic fungi—may be considered as of less importance and productive of little injury or loss under storage conditions.

Since the field-inoculation studies produced, on the whole, negative results, with the exception of those fungi listed in table 1, group A, the majority of the fungi would not be considered as serious parasites of the growing beet.

SUMMARY

About 5000 beets were examined for the presence of fungi. This included beets grown in the field and greenhouse, beets in storage, matured beets, and beet seedlings, and beets grown near Syracuse, N. Y., and from various sections of the United States.

Pure cultures were made of each of the fungi isolated.

The fungi thus isolated were divided into groups as follows:

A. *Parasitic fungi*

1. those of storage importance
2. those attacking the beet in the seedling stage

B. *Saprophytic fungi*

1. those producing little or no decay in stored beets
2. those of no economic importance, or the so-called secondary invaders.

Species of the genus *Fusarium* were responsible for most of the storage rots.

A number of fungi, including a great many species of *Fusarium*, are reported on beets for the first time.

Alternaria sp. is here reported for the first time as a parasite of beets in New York State.

Identification and description of about 50 species and varieties of the genus *Fusarium* are reported.

The role of *Phoma betae* as a parasite of beets is of minor importance among the fungi under these studies.

The parasitic importance of many of the species of *Fusarium* on growing beets has been shown to be negligible, and of little economic significance to the sugar beet industry.

Only a few of the fungi studied appeared to be active parasites and may be considered as important plant pathogens.

SYRACUSE UNIVERSITY
SYRACUSE, N. Y.

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A STUDY OF THE TOXIC ACTION ON GRAY-MOLD SPORES OF CLEANING SOLUTIONS USED IN SPRAY RESIDUE REMOVAL¹

OTTO F. SCHNELHARDT² AND F. D. HEALD

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INTRODUCTION

Apples, produced in the central irrigated valleys of Yakima and Wenatchee, are practically free from either decay or fungous lesions at the time of harvest, but storage diseases take a heavy toll of the harvested crop each year. Heald and Ruehle (8) recorded more than 40 species of fungi belonging to 22 genera as connected with decay of apples in Washington; of these blue mold and gray mold were probably the most destructive. Brien (3) in New Zealand found gray mold (*Botrytis cinerea*) causing an average of 16.83 per cent infection as compared to 5.6 per cent for *Penicillium expansum* on apples in cool storage. Examination³ of boxed apples in the Wenatchee area during the 1933-34 storage season indicate that gray mold may become destructive as a rot of stored apples wherever there is considerable rainfall during the harvesting period. Gray mold advances more rapidly in cold storage than blue mold. Fruit inoculated with pure cultures of the fungus may be completely rotted after two months at 0° C. or after two weeks at room temperature. *Botrytis* of the *cinerea* type is the most important gray-mold species causing decay of apples in Washington.

The fruit cleaning methods now in use consist of washing the fruit with various solvents. Hydrochloric acid or some of the alkaline compounds are the most common cleaners. These solutions are ordinarily heated in commercial practice to temperatures of 90° F. to 120° F. and occasionally as high as 130° F. The higher temperatures, however, often cause injury to certain varieties, either bleaching the skin or lowering the keeping qualities. During the last two seasons the types of washers previously used are being abandoned and new types adopted, largely on account of the use of tandem or double washes. When tandem washes are employed to clean fruit sprayed with lead arsenate, excellent cleaning may result with either the sequence of first, HCl, and second, sodium silicate, or the reverse. Other

¹ Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 314, College of Agriculture and Agricultural Experiment Station, State College of Washington, Pullman, Washington.

² Formerly Research Assistant in Plant Pathology, Washington Agricultural Experiment Station, Pullman, Washington.

³ Data collected by Mr. G. A. Newton while working on the apple rot survey in Washington.

alkaline cleaners, such as sodium carbonate-trisodium phosphate or soda ash, may be substituted for the sodium silicate when a milder alkali is desired.

Constant changes in methods and materials used in apple washing leads one to wonder what effect these alterations are having on the life of the fungous spores present in the washing and rinse tanks. Fruit-washing tanks have been found by various investigators to be a source of contamination by fungous spores. Heald *et al.* (7) in Washington, Pentzer in New York (10), Shear in the Hudson Valley (12), and Fisher and Reeves (6) found an accumulation of fungous spores in the cleaning and rinsing baths during commercial operations. The solvents used and the temperatures of the cleaning solutions in the earlier washing operations had little or no killing effect on the spores. Diehl *et al.* (5) stated certain rot-producing spores were able to survive exposure to either acid or alkaline washing solutions for 6 days. These spores originated from the surface of normal apples, from an occasional decayed apple that passed into the machine, and from the dust of the air.

In recent work Baker and Heald (1) have shown that temperatures of washing solutions within the ranges employed, exert a lethal effect on the spores of *Penicillium expansum*. The killing effect was markedly increased by addition of the washing compound. These two investigators (2) have further shown that treating the apples with a sodium hypochlorite rinse, following washing, is very effective in reducing the spore load of the surface of the fruit and the lenticel basins. It further decreased the percentage of decay at punctures and other points of entry.

MATERIALS AND METHODS

In this study on the toxic action of cleaning solutions the cultures used were transfers from the Botrytis isolated from decayed apples by Heald and Ruehle (8) and identified by them as *Botrytis cinerea* Pers. The conidia used were taken from vigorously growing 10 to 15-day-old colonies cultured on 2 per cent dextrose-potato agar.

The cleaners tried out experimentally in this work were those most commonly used in the packing plants during the 1933 and 1934 seasons. Hydrochloric acid at a strength of 3 per cent by volume was tested for the acid cleaner. In terms of this experiment it was 9 cc. per 300 cc. water blank. Two alkali cleaners were tested. The first of these was sodium carbonate at a concentration of 75 pounds per 100 gallons of water or 26.96 grams per 300 cc. water blank. This product was called Wyandotte soda ash, manufactured by the Michigan Alkali Company, Wyandotte, Michigan. The second alkali cleaner was sodium silicate used at the same concentration. This is called "B. W." silicate of soda and is manufactured by the Philadelphia Quartz Company of California, Ltd., Berkeley, California.

The methods employed were similar to those used by Baker and Heald

(1) with modifications better adapted for work with the gray-mold organism. In the tests with commercial hydrochloric acid as a cleaner, spores were scraped from Petri dish cultures and placed in a flask containing 100 cc. of spore-free water and shaken for 5 minutes to break up the spore clumps. Ten cc. of this concentrated spore suspension was transferred to flasks in triplicate containing 300 cc. sterile distilled water plus 9 cc. of the commercial HCl. A similar flask of water containing a spore suspension without the HCl was used as a check. These flasks were thoroughly agitated and 1 cc. from each was transferred with a sterile pipette to 9 cc. sterile water blanks, making a 1-10 dilution. Tests were made from these dilutions at timed intervals and controlled temperatures. The dilutions were placed on sterile Petri dishes with sterile pipettes and 2 per cent dextrose-potato agar cooled to about 42° C. poured over them and the dishes rotated. After 48 hours' incubation at room temperature the number of colonies per plate was counted, and an average for the three plates taken. From this count the number of gray-mold spores per cubic centimeter was calculated. Thus the figures (Table 2) are averages of the 3 plates made from each flask at each viability test and are expressions of the approximate number of viable gray-mold spores per cubic centimeter in the solution at the time the test was taken.

In the sodium carbonate tests (Table 3) the spore concentration was increased by transferring 20 cc. of the suspension to the flasks instead of 10 cc. Three of the flasks each contained 26.96 grams of soda ash plus 300 cc. sterile distilled water. One flask contained no washing compound and was used as a check. The dilutions were plated out in duplicate and an average of the 2 plates taken to determine the number of viable spores per cubic centimeter. The other procedure was the same as for the HCl tests.

The sodium silicate cleaner exhibited such a marked toxicity to the *Botrytis* spores that fewer tests were made, and the time intervals were shortened (Table 4). This required a rapid transfer from the flasks to the dilution tubes when the cleaners were tested at the higher temperatures. Only 2 flasks were used in this series. One contained 26.96 grams of sodium silicate, and the other without the cleaner was used as a check. These were tested at one-half and one minute intervals.

The effect of these cleaners on spore survival was tested at room temperature (68-70° F.), 90° F., 100° F., 110° F., and 120° F. In order to obtain the effect of the solutions at different temperatures the flasks were placed in an incubator. The flasks were placed in pans of water while being held in the incubator to stabilize the temperature and to reduce the evaporation of the solutions that would have increased the concentration of the treating compound.

The temperatures of the flasks were stabilized by keeping them in an incubator overnight at the desired degree, after which the spores were added. Transfers from the flasks were made in a sterile culture chamber and performed as quickly as possible. During this time the flasks were held in the water pans and the temperature fluctuation was slight.

Commercial apple-washing practices were duplicated as nearly as possible in these laboratory tests. The spores in suspension in the flasks were subjected to both the lethal effect of the solute and to that of the temperature. This is similar to the conditions in the packing plant, where the fungous spores are in a heated washing tank in the presence of a cleaner. Thus, the results obtained would give some indication of the toxicity of these cleaners at the temperatures employed. These in turn could be used as indices to determine the lethal point of gray-mold spores. Spore viability was determined by the ability to form colonies on 2 per cent dextrose-potato agar plates.

The degree of experimental variation was determined (Table 1). Four flasks of 300 cc. sterile distilled water were inoculated with a spore suspension and 10 plates poured at once from each flask, using 1 cc. of a 1-10 dilution. At times the range of experimental variation appeared fairly broad, but this probably was due to the variable number of viable spores transferred with the 1 cc. pipette to the 9 cc. water blank.

The subsequent transfer of the dilutions to the sterile Petri dishes would again vary somewhat because the number of spores in each dilution would show some deviation. The average of the plates poured, within certain limits, showed considerable uniformity of results.

TABLE 1.—Possible experimental variation of viable spores of *Botrytis* per cubic centimeter from the same spore suspensions

Plate ^a	Flask 1	Flask 2	Flask 3	Flask 4
1	1370	1230	780	970
2	1270	1540	1030	1280
3	1120	1470	970	1170
4	1060	1280	1000	1480
5	1370	1620	1220	1330
6	1070	1560	1010	1350
7	1030	1580	900	1190
8	980	1330	960	1050
9	1340	1530	820	1170
10	1330	1420	1040	1280
Ave.	1194	1456	973	1227

^a One cc. of spore suspensions placed in 9 cc. sterile water blanks from which 1 cc. was removed to each poured plate.

THE EFFECT OF CLEANING SOLUTIONS ON GRAY-MOLD SPORES
AT DIFFERENT TEMPERATURES

The effect of hydrochloric acid on gray-mold spores at the usual strength of commercial cleaning and at various temperatures was determined (Table 2). This cleaner exerted a marked toxic effect on gray-mold spores, which increased with increased temperature and longer exposures. At room temperature 81.3 per cent of the spores were killed at the end of 104 hours with no reduction occurring in the water check after this time. The reduction in number of gray-mold colonies resulting from the action of the cleaner after 72 hours at room temperature is shown in (Fig. 1, A, D). At 90° F. in the water check, 55.6 per cent of the spores were killed after 60 hours, while in the cleaner they were all dead after 25–36 hours. At 100° F. the water check showed a 54.8 per cent kill after 24 hours and a complete kill after 7 hours in the cleaner. The spores were all killed after 12 hours' exposure in the water check at 100° F. and viability ceased after 2 hours in the cleaner. At 120° F. all spores were killed after 35 minutes in the water check and the lethal point was reached in 15 minutes with the addition of the cleaner.

The sodium carbonate cleaner (Table 3) showed a marked effect on the Botrytis spores at 90° F. and at room temperature. A 100 per cent kill and a 98.8 per cent kill, respectively, took place at these two temperatures after 101 hours, while the water checks showed no reduction in number of colonies. At 100° F. the soda ash gave a kill of 99.6 per cent as compared to 52.2 per cent for the water check after 36 hours, while at the end of 48 hours viability ceased in both the water check and the cleaner. The reduction in number of colonies on account of the soda ash treatment at 100° F. is well shown in the illustration (Fig. 1, B, E). When subjected to a temperature of 110° F. for 11 hours, the spores were killed in both the water check and the cleaner, while in the cleaner there was a nearly complete kill after 7 hours. In flasks held at 120° F. the spores were all killed in 20 minutes as contrasted to 30 minutes for the water check.

Sodium silicate had the greatest effect on the viability of gray-mold spores. No reduction was noticeable in spore number in the water checks of this series because of the short time the spores were held therein. The flasks of cleaner showed a greater reduction in spore number as temperature and time of exposure were increased. The results (Table 4) show a 95.5 per cent kill at room temperature after 5 minutes; a complete kill at 90° F. after 5 minutes; all spores dead after 3 minutes at 100° F.; no viable spores after 2 minutes at 110° F.; and a complete kill in 1 minute at 120° F. Reduction in colony number by exposure at 90° F. for 3 minutes is shown in figure 1, C, F.

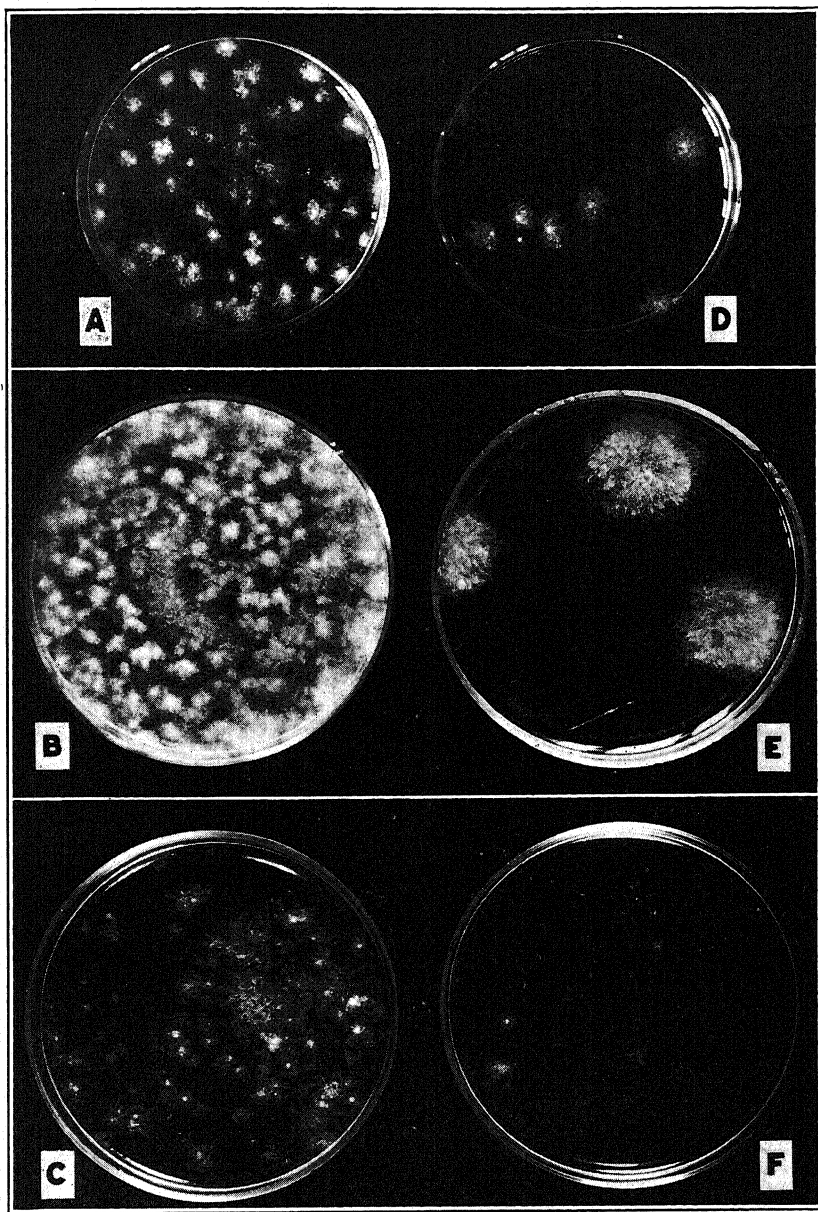


FIG. 1. Colonies of *Botrytis cinerea* on 2 per cent potato-dextrose agar. A-C. Water checks. D. From flask held 72 hours at room temperature in 1 per cent HCl. E. From flask held 30 hours at 100° F. in soda ash cleaner. F. From flask held 3 minutes at 90° F. in sodium silicate cleaner.

TABLE 2. *Number of viable gray-mold spores per cubic centimeter obtained at intervals from spore suspensions in 3 per cent HCl at different temperatures*

Room temperature 68-70° F.				
Time exposed	Water check	3% commercial hydrochloric acid		
		Flask 1	Flask 2	Flask 3
0 hours	2413	2360	2173	1813
12 "	2240	1347	1800	1493
24 "	2267	1387	933	1960
36 "	2173	823	423	1027
48 "	2120	627	507	1067
60 "	2560	680	1133	927
72 "	2253	720	440	867
96 "	2707	707	280	773
104 "	2187	413	253	587
90° F. temperature				
0 hours	2400	2360	1907	2560
8 "	2293	93	97	90
12½ "	2653	47	20	70
25 "	2347	0	10	0
36 "	2707	0	0	0
49½ "	2027	0	0	0
60 "	1067	0	0	0
100° F. temperature				
0 hours	4227	4480	4693	4467
1 "	5400	3040	4147	3760
3½ "	2333	0	13	0
7 "	4680	0	0	0
12 "	3353	0	0	0
24 "	1913	0	0	0
110° F. temperature				
0 hours	4253	3973	4133	4187
1 "	2827	1920	1307	573
2 "	3440	0	0	0
3 "	4493	0	0	0
5 "	3800	0	0	0
7 "	3503	0	0	0
12 "	0	0	0	0
120° F. temperature				
0 minutes ...	4600	4040	7600	8520
5 " ...	5060	160	20	1860
10 " ...	4500	0	0	20
15 " ...	1120	0	0	0
20 " ...	60	0	0	0
25 " ...	40	0	0	0
30 " ...	20	0	0	0
35 " ...	0	0	0	0

TABLE 3. *Number of viable spores of gray mold per cubic centimeter obtained at intervals from spore suspensions in soda ash cleaner, 75 lbs. per 100 gals., at different temperatures*

Room temperature				
Time exposed	Water check	Sodium carbonate (soda ash)		
		Flask 1	Flask 2	Flask 3
0 hours	5840	5660	5920	6660
12 "	8500	860	1580	1400
24 "	7480	20	880	680
48 "	6140	0	680	520
60 "	4880	0	420	360
72 "	6160	0	440	260
84 "	5260	0	160	80
101 "	6460	0	100	40
90° F. temperature				
0 hours	6160	8400	6820	6300
12 "	9200	4480	5900	4840
24 "	8300	1080	5980	4420
48 "	9860	80	1440	760
60 "	8500	80	600	280
72 "	7280	0	140	40
84 "	7040	0	20	20
101 "	6400	0	0	0
100° F. temperature				
0 hours	13960	12520	16160	14240
8 "	11680	14940	3100	16520
14 "	13200	2380	120	3400
24 "	10440	380	0	440
30 "	8880	160	0	120
36 "	6680	60	0	60
48 "	0	0	0	0
60 "	0	0	0	0
110° F. temperature				
0 "	11680	9440	8080	8960
2 "	4480	840	4260	2340
4 "	1340	100	1060	320
7 "	640	0	20	20
11 "	0	0	0	0
13 "	0	0	0	0
120° F. temperature				
0 minutes ...	4920	4400	4500	3640
10 " ...	3860	0	2560	3080
20 " ...	40	0	0	0
30 " ...	0	0	0	0
40 " ...	0	0	0	0

TABLE 4.—*Number of viable spores of gray mold per cubic centimeter obtained at intervals from spore suspensions in sodium silicate 75 lbs. per 100 gals., at different temperatures*

Room temperature					
Time exposed	Water check	Sodium silicate	Time exposed	Water check	Sodium silicate
0 minutes	5400	5080	3 minutes	14800	4780
1 "	10420	9200	4 "	10900	1500
2 "	9750	7180	5 "	8000	360
90° F. temperature					
0 minutes	9350	3600	3 minutes	9200	400
1 "	8100	3460	4 "	13400	320
2 "	8100	1700	5 "	20400	0
100° F. temperature					
0 minutes	12900	3240	3 minutes	13900	0
1 "	15800	200	4 "	13000	0
2 "	12200	20	5 "	17700	0
110° F. temperature					
0 minutes	7700	2140	1½ minutes	9900	20
½ "	6650	160	2 "	8650	0
1 "	4450	20	2½ "	9450	0
120° F. temperature					
0 minutes	3750	180	1½ minutes	3750	0
½ "	7500	20	2 "	7650	0
1 "	7700	0	2½ "	7050	0

In plates made from the suspension of spores held in the water check for 2 hours at 110° F., table 2, colonies of limited size were formed in one plate in contrast to the normal spreading type (Fig. 2, A, B). The same type of colony formation appeared again in duplicate plates from one of the 3 per cent HCl flasks after 24 hours at room temperature (Fig. 2, C, D); but there were fewer colonies per plate and the colonies of limited size grew larger. The same phenomenon occurred again in the 3 per cent HCl series after 1 hour at 110° F. These 3 instances were the only ones observed in all the plates poured.

To determine whether the temperature factor was responsible for the abnormal colony formation, suspensions of spores from normal colonies were made and plates poured at different temperatures from 43° C.-73° C. at 5-degree intervals of temperature increases. The colony formation of limited size was not observed in these plates. Spore suspensions were made from these peculiar colonies and plated out, but the abnormal type could not be reproduced. From these tests this colony formation of limited size can be explained neither from the temperature factor nor on the basis of a

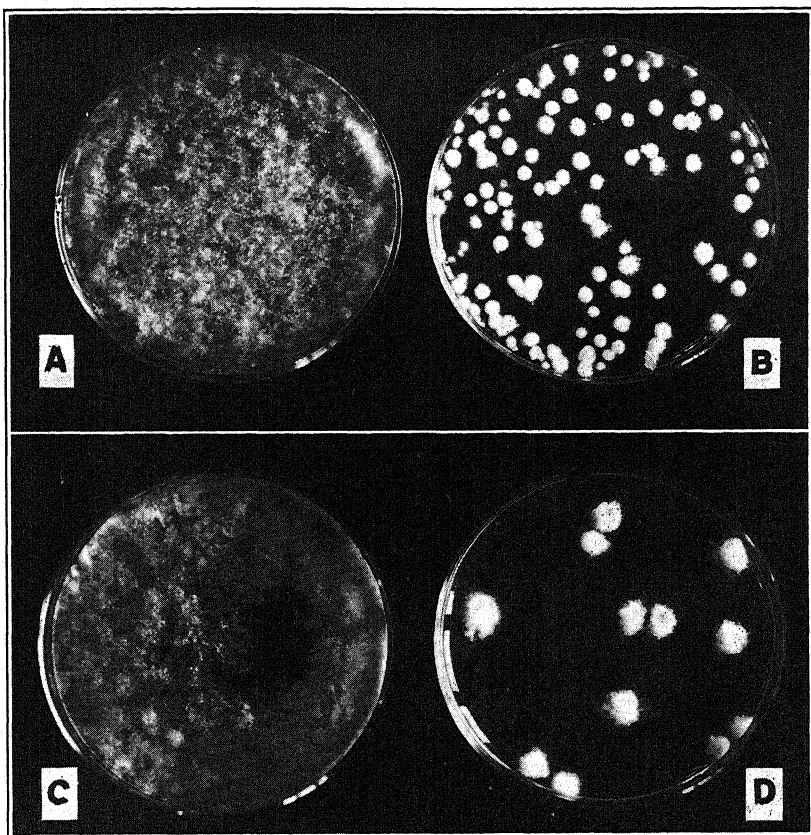


FIG. 2. Six-day-old colonies of *Botrytis cinerea* on 2 per cent potato-dextrose agar. A. Normal colonies. B. Abnormal colonies of limited size from water-check flask held 2 hours at 110° F. C. Normal colonies. D. Abnormal colonies of limited size from HCl flask held 24 hours at room temperature.

mutant. It appears to be due to some factor or condition entering into the methods used, the cause of which could not be determined.

DISCUSSION

Apple washing for the removal of spray residue by one of the chemical or wet processes has been practiced since 1927. Commercial hydrochloric acid, 1 per cent by weight or 3 per cent by volume, is most commonly used in apple washing. Various alkaline solvents have been tested, and those used most widely in recent years in this State are sodium carbonate and sodium silicate at concentrations of 75 to 100 pounds per 100 gallons of water. Recent washing experiments in Washington (9) and Oregon (11) have given good results with a double process wash, which consists of passing the fruit

through a washing unit containing sodium silicate and then through a hydrochloric acid wash, or the reverse. Robinson and Hatch (11) found hydrochloric acid was most effective at relatively low concentrations and at 100° to 110° F. They report sodium silicate a slower acting solvent than hydrochloric acid and recommend its use at higher concentrations, longer periods of time in the solvent, and at higher temperatures (110° to 120° F.). Overley *et al.* (9) report sodium silicate at a concentration of 80 to 100 pounds per 100 gallons of water at a temperature of 120° F. effective in the reduction of both lead and arsenic. A good foam was maintained in the solution and underneath brushes were used. Sodium silicate appeared to be more effective in removing the lead than soda ash. Baker and Heald (2) give data to show that sodium silicate reduced blue-mold decay to a greater extent than the other washing solutions tested. They do not recommend sodium silicate as an effective prevention of decay of apples if the inoculum is at a high level in the washing and rinsing tanks. In order to remove the residue the apples must remain in the solvent from 1 to 3 minutes, while the time required for rinsing ranges from 40 seconds to 1 minute. Temperatures of 100° F., 110° F., and 120° F. have been found most effective for the solvent bath.

Baker and Heald (1) have shown that both acid and alkaline cleaners, as used in commercial practice, killed off nearly all the blue-mold spores after an overnight stand. They also showed that the cleaners used were toxic to *Penicillium expansum* spores after shorter periods of exposure, and that the temperature alone, as well as the solvent, had a lethal effect on these spores.

In this investigation a study was made of the effect these cleaners have on gray-mold spores. The effect of both temperature and cleaners is summarized in table 5.

The cleaners were toxic on gray-mold spores at room temperatures and, as the solution were heated, the lethal effect on the spores increased with the rise in temperature.

The results (Table 5) indicate that the orchardist or fruit packer need have little concern about gray-mold contamination in his washing tank when using sodium silicate, at least when operating at the higher temperatures. This, however, would not protect against spores in the air or those present in the packing house. Chemically treated wraps have been found of commercial value in the prevention of botrytis rot of pears by Cooley and Crenshaw (4), while iodized wraps have shown promise in reducing some storage rots of apples (Tomkins, 13). This phase was not investigated for botrytis rot of apples.

When hydrochloric acid alone is used there might be a slight chance of contamination at the lower temperatures. The gray-mold spore load was

TABLE 5.—*Summary of the effect of cleaning solutions and of water at different temperatures on gray-mold spores*

Temperature	Per cent of spores killed after exposure stated							
	Per cent	Hrs.	Per cent	Hrs.	Per cent	Hrs.	Per cent	Min.
	Water Check		Hydrochloric acid (1 per cent by wt.)		Sodium carbonate 75 lbs. per 100 gals.		Sodium silicate 75 lbs. per 100 gals.	
Room	0.0	101	81.3	104	98.8	101	95.5	5
90° F.	0.0	101	99.9	25	99.9	84	52.7	2
			100.0	36	100.0	101	100.0	5
100° F.	52.2	36	99.9	3.5	99.9	36	98.7	1
	100.0	48	100.0	7.0	100.0	48	100.0	3
110° F.	94.6	7	69.1	1	99.9	7	97.6	0.5
	100.0	11	100.0	2	100.0	11	100.0	2
120° F.	Per cent	Min.	Per cent	Min.	Per cent	Min.	Per cent	Min.
	99.9	30	99.9	10	55.1	10	99.9	0.5
	100.0	35	100.0	15	100.0	20	100.0	1

greatly reduced by a heated solution and when followed by a running water rinse there should be very little gray-mold infection.

Sodium carbonate showed the least toxicity of any of the cleaners tested. At the common temperature of 110° F. there was considerable danger of contamination, if any large amount of gray-mold inoculum were present. Even at 120° F. the inoculum might accumulate from the spore load of the new apples or from an occasional decayed apple being introduced into the washing tank.

The introduction of tandem or double process washes and the widespread use of sodium silicate should greatly reduce mold contamination in the washing tanks. Washing with higher temperatures would have a tendency to reduce the spore load. A double wash at a temperature less than 100° F. with an alkali other than sodium silicate may offer some chance for gray-mold contamination. Observations⁴ of commercial fruit in Yakima in 1934 indicate that temperatures above 120° F. injure the apples and open up avenues of entry for decay-producing fungi, since greater percentages of decay, mostly blue mold, were recorded from the apples washed at the higher temperatures ranging from 120° to 130° F. Gray mold, ranging from 0.2 to 1.4 per cent, occurred in 6 of the lots examined. This would indicate that some of the spores survived the action of the cleaning solutions at these

⁴ Unpublished data collected by Paul Allen, former student in the Department of Plant Pathology.

temperatures. It also is possible that they were lodged in the lenticel basins, calyx, or stem cavities and did not come in direct contact with the cleaner.

SUMMARY

Washing tanks are one of the main sources of contamination of apples with gray mold, and it is desirable to find the most feasible sanitary practices to reduce this contamination.

The washing tank becomes contaminated with gray-mold spores from the surface of normal apples, from an occasional decayed apple that passes into the machine, and from the dust of the air.

The toxic action of hydrochloric acid, sodium carbonate, sodium silicate, and water on gray-mold spores was determined. Temperatures of the solutions ranged from room to 120° F.

Spores of *Botrytis cinerea* were held in suspension in flasks and subjected to both chemical toxicity and temperature fluctuations. Spore viability was ascertained by the ability to form colonies on 2 per cent dextrose-potato agar.

Abnormal gray-mold colonies of limited size developed in contrast to the normal spreading colonies when duplicate plates were made from the same flask. The cause of this phenomenon was not determined.

Cleaning solutions used in the removal of spray residue have a toxic effect on gray-mold spores. Of the 3 cleaners commonly used in the removal of spray residue, sodium silicate was the most toxic to gray mold, hydrochloric acid less toxic, and sodium carbonate the least so. Toxicity increased with a prolongation of exposure to the chemical and a rise in temperature.

The temperatures at which the cleaning solutions were used had a marked toxic action on gray-mold spores. As the solutions were heated from 90° F. to 120° F., the percentage of spores killed increased.

The results indicate that the orchardist or fruit packer need have little concern about gray-mold contamination during the washing operations when using sodium silicate, at least when operating at the higher temperatures.

The introduction of tandem or double process washes at the higher temperatures and the widespread use of sodium silicate greatly reduces the chances of gray-mold contamination in the washing tanks.

DEPARTMENT OF PLANT PATHOLOGY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON.

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STUDIES ON THE EFFECT OF CARBORUNDUM AS AN ABRASIVE IN PLANT VIRUS INOCULATIONS¹

T. E. RAWLINS AND C. M. TOMPKINS²

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INTRODUCTION

Some viruses, such as the tobacco mosaics and cucumber mosaic viruses may be readily transmitted by rubbing infective juice on the leaves of susceptible hosts, as recommended by Holmes (10), but many have been regularly transmitted only by insects or grafts. One of the most important viruses, difficult to transmit regularly by the use of Holmes' method, is the spotted-wilt virus, which infects a number of hosts but is particularly destructive to tomato and lettuce. Other viruses that have not been transmitted regularly by the rubbing method are the sugar-beet, crucifer, broad-bean, and celery-mosaic viruses, all of which occur in California.

Failure to regularly transmit these viruses may be due to failure of the inoculation methods to produce minute openings in the host cell walls. It is supposed that very small openings in the host cells admit virus particles but do not injure the cells to such an extent as to render them unfavorable for virus multiplication. Evidence in favor of this hypothesis has been furnished by workers who have used sand as an abrasive. Fajardo (6) found that sand rubbed on the leaves with the bean-mosaic virus increased infection; and Fajardo and Maranon (7) used this method successfully in transmitting mosaic of Sincamas, *Pachyrrhizus erosus* L. Urban. Samuel and Bald (18) found that 120-mesh quartz river sand added to a suspension of the yellow tobacco-mosaic virus greatly increased the number of primary lesions produced on various species of *Nicotiana*.

It, therefore, appeared reasonable to assume that certain other abrasives composed of smaller and sharper crystals might be more satisfactory than sand in producing the supposedly necessary minute openings in inoculated cells. It was found that powdered carborundum crystals possessed the desired structure. A note on the use of carborundum has already been published (16). Further experiments carried out to test this hypothesis are described in detail.

MATERIALS AND METHODS

Young, healthy plants, grown from seed in the greenhouse and later transplanted to 6-inch porous clay pots, were used in all inoculation experi-

¹ Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

² The writers are indebted to Professor Ralph E. Smith and Dr. Max W. Gardner for helpful suggestions and advice.

ments. The greenhouse temperature varied from 55° to 65° F. Weekly fumigation of the greenhouse was given by heating a small quantity of nicotine sulphate in a shallow pan. This gave good control of aphids and thrips.

In preliminary trials various abrasives, such as finely ground but ungraded quartz sand, powdered silicic acid and jewelers' rouge, were tested. Since the results obtained by the use of these substances did not seem promising, further tests were limited to a 600-mesh, powdered carborundum. This substance was dusted over the upper surface of the leaves to be inoculated by means of a salt shaker provided with small openings. No attempt was made to distribute the abrasive uniformly when applying, since more or less uniform distribution resulted later when the virus suspension was wiped on the leaf.

Virus suspensions were prepared by grinding leaves from young plants showing the first symptoms of disease in a mortar lined with a square of cheesecloth. After grinding to a pulp the juice was pressed through the cheesecloth. Except where noted later, the extracted, undiluted juice was applied without centrifuging or purifying.

A sterile, 8-inch pot label was held under the leaf to be inoculated. A small ball of sterilized absorbent cotton, held in forceps, was dipped into the virus suspension and then twice drawn lightly over the leaf surface in one direction, usually from the base to the tip of the leaf. This operation tended to distribute the particles of carborundum and virus more or less uniformly over the leaf surface, but did not cause any macroscopic injury to the leaf tissues. Conspicuous injury does occur if the carborundum is rubbed into the leaf by heavy pressure on the cotton. All the leaves on each test plant, usually 2 or 3 in number, were inoculated.

Experimental

In studying the efficacy of carborundum for rendering plants susceptible to virus infection, a number of diseases were investigated in order to determine how generally the method may be applied. Certain experimental details and the results are given in table 1.

Spotted Wilt

This virus was transferred from lettuce to lettuce and from lettuce to tomato. As is shown in table 1, the use of carborundum during inoculation gave a very significant increase in infection of both hosts.

The spotted-wilt virus produces primary local lesions around the leaf cells where infection has occurred in the case of *Nicotiana glutinosa*. Leaves of this plant were inoculated in the usual way, with and without carborundum. As is shown in table 2, although enormous variation is observed

TABLE 1.—*Transmission of different viruses with and without the use of carborundum*

Virus used	Source of inoculum	Host inoculated	Date inoculated	Date read	Mode of inoculation			
					Carborundum		No carborundum	
					Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected
Spotted-wilt	Diseased Romaine lettuce plant, naturally infected. San Pablo, California	Lettuce	4-18-34 5-1-34 12-31-34	5-4-34 5-21-34 1-20-35	20 20 20	8 18 14	20 20 20	0 4 0
Spotted-wilt	"							
Broad-bean mosaic	Diseased broad-bean plant, naturally infected. Niles, California	Tonato	12-24-34	1-20-35	20	16	20	6
		Broad bean	5-31-34 7-19-34a	6-16-34 8-6-34	44 27	31 19	39 32	1 7
Celery-mosaic	Diseased celery plant, artificially infected with a celery-mosaic virus from Southern California	Celery	10-8-34 10-31-34	10-23-34 11-15-34	25 24	23 15	25 23	3 3
Cauliflower-mosaic	Diseased cauliflower plant, naturally infected. Alvarado, California	Cauliflower	6-5-34 12-20-34	6-20-34 1-8-35	20 25	15 23	20 25	0 0
Sugar-beet mosaic	Diseased sugar beet, naturally infected. Berkeley, California	Sugar beet	8-23-34 10-16-34	9-7-34 10-29-34	70 50	50 37	70 50	17 14
California-aster or celery-yellows	Diseased aster plant, experimentally infected. Berkeley, California	Aster	10-8-34 11-8-34	11-20-34 12-10-34	25 20	0 0	25 20	0 0

^a 13 noninoculated broad-bean plants, used as controls, remained healthy.

TABLE 1.—Continued

Virus used	Source of inoculum	Host inoculated	Date inoculated	Date read	Mode of inoculation			
					Carborundum		No carborundum	
					Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected
Lettuce brown-blight ^b	Diseased lettuce plants, naturally infected. Salinas, California	Lettuce	10-22-34 11- 1-34	11-22-34 12- 1-34	20 20	0 0	20 20	0 0
Lettuce big-vein ^b	Diseased lettuce plants, naturally infected. San Pablo, California	Lettuce	3-17-34 4-25-34	4-20-34 6- 2-34	15 20	0 0	15 20	0 0
Sugar-beet curly-top	Diseased sugar-beet leaves from naturally infected plant. San Ardo, California	Sugar beet	6- 9-34	7-16-34	25	0	25	0
	Diseased sugar-beet root from naturally infected plant. San Ardo, California		6-30-34	8- 7-34	25	0	25	0
	Diseased sugar-beet root from naturally infected plant. San Ardo, California		8-14-34	9-21-34	25	0	50	0

^b Not yet definitely identified as virus diseases.

in the results, the carborundum caused a greatly increased total number of local lesions.

TABLE 2.—*Inoculations of Nicotiana glutinosa*,^a with and without carborundum, with the spotted wilt virus

With carborundum		No carborundum	
Plant no.	No. local lesions	Plant no.	No. local lesions
1	2	17	0
2	4	18	0
3	21	19	0
4	12	20	8
5	12	21	1
6	4	22	5
7	7	23	3
8	58	24	12
9	7	25	3
10	6	26	1
11	6	27	1
12	106 ^b	28	4
13	10	29	2
14	7	30	2
15	13	31	5
16	165	32	1
Totals	440 [†]		48 ^{††}

^a Inoculated 12-5-34. Read 12-13-34.

^b Systemic infection. Plant died on the 8th day.

[†] Average number local lesions per plant when carborundum was used = 27½.

^{††} Average number local lesions per plant when carborundum was not used = 3.

Broad-bean Mosaic

Studies of the mosaic of broad-bean in Germany by Böning (3) and in Japan by Fukushi (8) and Imai (11) have shown the difficulties encountered in trying to obtain infection by mechanical means. Böning did not succeed in inducing the disease by artificial means, while Imai secured only 34 per cent infection.

As shown in table 1, a high percentage of infection resulted only when carborundum was used, demonstrating the efficacy of this material as an aid in transmitting broad-bean mosaic.

A California Celery Mosaic

The celery-mosaic virus with which we worked was obtained from diseased celery plants collected at Venice, California. This virus was recently described by Severin and Freitag (20). As is shown in table 1, this disease is

difficult to transmit regularly by Holmes's method. The carborundum method increased the percentage of transmission approximately 5 to 7 times.

Cauliflower Mosaic

This recently described virosis, (22) appears to be different from any of the crucifer viruses previously reported in the literature. As is shown in table 1 it was not transmitted by Holmes's method but was transmitted to 38 of the 45 inoculated plants by means of the carborundum method. Transmission has never been obtained by rubbing without the carborundum.

Sugar-beet Mosaic

Robbins (17) and Böning (2) failed to infect healthy sugar beets with the virus of sugar-beet mosaic by juice inoculations. Jones (14) tried unsuccessfully to transmit the mosaic disease of garden beet by mechanical means. Verplancke (23) and Smith (21) secured transmission by rubbing, while Hoggan (9) reported systemic infection as a result of severe rubbing.

Our preliminary test with the rubbing method also gave negative results. In the first test with carborundum, 71 per cent of the plants were infected as compared with 24 per cent where the abrasive was omitted. In the second test, 74 per cent infection with carborundum, as against 28 per cent without, was obtained. Thirty healthy noninoculated sugar-beet seedlings served as controls in each series to test the possibility of accidental infection. All control plants remained healthy. It is, therefore, evident that this virus may be readily transmitted by the use of carborundum.

Aster Yellows³

All workers who have attempted to transmit the two strains of aster yellows by juice inoculations have been unsuccessful. As is evident from the results shown in table 1, the carborundum method also was unsatisfactory in transmitting the California-aster or celery-yellows virus. It is possible that this virus, like the curly-top virus, is confined to the phloem and that any successful method of inoculation must involve the injection of the virus into the phloem.

An attempt was made to introduce the virus into the phloem by cutting away the lower portion of the midrib and larger veins with a sharp razor blade to expose the phloem and then rubbing the cut tissues with 600-mesh carborundum and virus suspension by means of a glass spatula. Five aster plants were thus inoculated but no infection resulted in any of the plants.

³ Grateful acknowledgment is made to Dr. H. H. P. Severin and Dr. Julius H. Freitag for having supplied the curly-top and aster-yellows viruses, respectively.

Curly Top⁴

Numerous attempts have been made to develop a satisfactory method of mechanical inoculation for the transmission of the curly top virus. Tests were made by Carsner and Stahl (4), Severin (19), and Dana (5), but only a very small percentage of infection resulted, so that the principal means of transmission continues to be that of the insect vector.

After inoculation with the curly-top virus by the carborundum method, sugar-beet seedlings in each of 3 series were held for 5 weeks; none of the plants became infected. The negative results were not wholly unexpected, since Bennett (1) has shown that the path of the virus is in the phloem. It is believed that no carborundum particles reached the phloem, which may account for lack of infection.

As in the case of the California aster or celery yellows, an attempt was made to introduce the virus into the phloem of beet leaves by cutting away the lower portion of the midrib and larger lateral veins to expose the phloem and then rubbing virus and 600-mesh carborundum over the cut tissues by means of a glass spatula. Fifteen beet plants were inoculated by this method but none of the plants became infected.

Brown Blight of Lettuce

Jagger (12) found brown blight of lettuce to be soil-borne but he was unable to transmit it by other means. Since the symptoms of this disease suggest that it may be a virosis an attempt was made to transmit it by means of the carborundum method. As is shown in table 1, infection did not result from any of the inoculations.

Big-vein Disease of Lettuce

Jagger and Chandler (13) have recently reported that big vein of lettuce also is soil-borne. Since this disease also exhibits symptoms occurring in some viroses an attempt was made to transmit the disease to healthy lettuce plants by means of the carborundum method. As is evident from the results shown in table 1, all of the inoculations were unsuccessful.

Histological Studies

An attempt was made to gain evidence regarding the action of the carborundum particles on the host cells by cutting sections of cauliflower leaves that had been inoculated with cauliflower mosaic virus after the leaves had been dusted with carborundum powder. The inoculated tissues were fixed at times varying from 1 to 29 hours after inoculation; Karpechenko's solution and Flemming's strong solution diluted with equal parts of water were

⁴ See footnote 3.

found to be the most satisfactory fixing solutions. After fixation the tissues were dehydrated with glycerin and infiltrated in turn with butyl alcohol and paraffin, as previously reported (15). When sectioning, the block was always so oriented that the microtome knife would cut through the leaf before striking any of the crystals on the upper surface of the leaf. This was done to insure that no crystals should be carried into the leaf cells by the knife. The sections were cut rather thick ($15\ \mu$ to $20\ \mu$) in order that minimum disturbance of the carborundum crystals by the knife should result. The sections were stained with Heidenhain's iron alum-hematoxylin combination. In certain cells the polarizing microscope was found to aid in observing the penetration of the cell walls by the crystals.

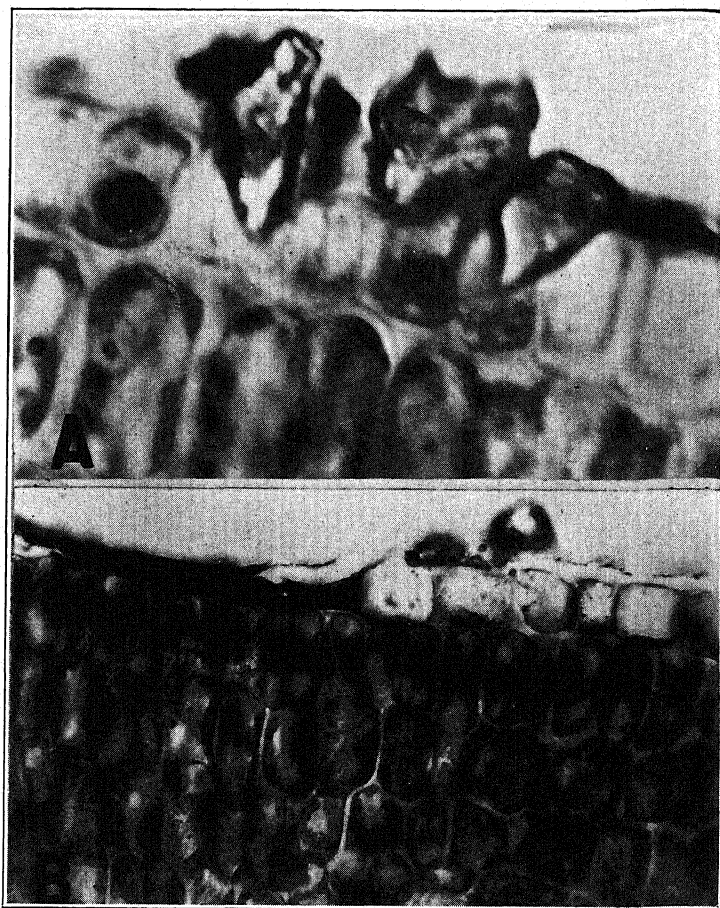


FIG. 1. Sections of cauliflower leaves on which carborundum was used during inoculation. A. Crystals that have pierced the epidermal cells. B. Collapsed epidermal cells, killed by the carborundum.

As is seen in figure 1, A, numerous epidermal cells that apparently had been penetrated by the carborundum crystals were observed. It is also evident that the points of the crystals are often small relative to the size of the cells and should therefore be able to penetrate the cells with a minimum of injury. In certain cases, as shown in figure 1, B, some of the epidermal cells were killed by the carborundum and have collapsed.

Since the histological work has shown that epidermal cells are frequently pierced by the sharp points of the crystals, it is reasonable to suppose that during the inoculation procedure some of the carborundum crystals produce small openings that allow virus particles to enter but do not injure the cells sufficiently to prevent virus multiplication.

SUMMARY

By the use of powdered carborundum during inoculation, it was possible to transmit readily a number of viruses that have been found difficult to transmit by other methods. Among these viruses are those causing spotted wilt of tomato and lettuce, broad-bean mosaic, a California celery mosaic, cauliflower mosaic and sugar-beet mosaic. Attempts to transmit curly top of sugar beet, California aster or celery yellows, and brown blight and big vein of lettuce were unsuccessful.

The 600-mesh grade of carborundum apparently was effective.

Histological studies demonstrated that in leaves inoculated by the carborundum method the epidermal cells frequently are pierced by the carborundum crystals and that the points of the crystals are small relative to the size of the cells. This evidence favors the hypothesis that the carborundum crystals may pierce leaf cells and allow virus particles to enter but in certain cases do not injure the pierced cells sufficiently to prevent virus multiplication in these cells.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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THE INFLUENCE OF SEED HULLING ON LOOSE SMUT IN NATURALLY INOCULATED OATS

V. F. TAPKE

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INTRODUCTION

In studies on the loose smut of oats (*Ustilago avenae* (Pers.) Jensen) the methods of artificial seed inoculation commonly employed in the past have consisted chiefly in applying dry spores to the surface of nonhulled or hulled¹ seed as noted in the review by Stanton *et al.* (9). In 1922, however, Zade (10) first showed that, under natural conditions of inoculation, the effective inoculum is chiefly mycelium that invades the inner parenchymatous layers of the hulls. This mycelium results from the germination of spores carried within the flowers during anthesis. The principal facts of Zade's discovery have since been repeatedly confirmed (1, 2, 3, 7, 8, 11). A striking difference, therefore, exists between the artificial and natural methods of inoculation with respect to the relative positions of inoculum and hulls. In the artificial methods, the inoculum comes into direct contact with the soil when the seed is sown. The hulls can play no part in shielding it against the full force of conditions inimical to infection. In the natural method of inoculation, however, the inoculum is protected by the enclosing hulls. Considering their siliceous nature and tight appression to the caryopsis, it would seem that they might function importantly in the case of naturally inoculated oats in protecting the inoculum against desiccation, excess moisture, sudden changes in temperature, and other unfavorable soil factors, during the critical period of seed germination and seedling infection. The influence of the hulls in this respect apparently has not been determined; obviously, a factor that might make an important difference in the results obtained from artificial and natural methods of seed inoculation. A study of the problem, therefore, seemed desirable. The procedure in the following experiments involved the hull removal of naturally inoculated seed. Further opportunity, therefore, was afforded to determine whether the effective inoculum resided chiefly in the inner parenchymatous lining or other parts of the hulls or in the pericarp of the caryopsis. As noted later, previous investigators are not in accord on this point. The results of the writer's investigation are presented in the following pages.

RELATED INVESTIGATIONS

In the loose smut of oats, the diseased panicles emerge and shed their smut spores during the emergence and anthesis of the normal smut-free

¹ In the present report, the term *hulled* signifies that the hulls have been removed from the mature seed by hand. The term *nonhulled* refers to oat seed with the hulls intact.

heads. Floral inoculation of the host commonly occurs throughout this period consequent upon the opening of the glumes and exposure of the flowers to errant smut spores.

Previous to Zade's discovery in 1922 (10), the importance of the positional relation of seed inoculum to smut infection in oats was not realized. Since 1922, however, Zade (11), Arland (1), Diehl (2), Roesch (7), Gage (3), and Sampson (8) have further contributed, showing that spores on the outside of the hulls play a minor part in causing loose smut in oats under natural conditions of inoculation. Their research also has shown that the spores entrapped within the glumes do not remain dormant, as formerly believed, but normally begin to germinate within a few hours and eventually give rise to a mycelium that invades various structures of the seed. Zade and Arland (13) concluded that under their ecological conditions, in Leipzig, Germany, chiefly the parenchyma of the hulls is invaded by the mycelium.

Gage (3) found that removal of the hulls of seed from hand-inoculated flowers effected only a small reduction in the percentage of smut. He concluded, therefore, that the effective inoculum resulting from floral inoculation consists in mycelium that eventually invades chiefly the pericarp of the caryopsis. Johnston (6), however, obtained high reductions in the amount of smut in 5 naturally inoculated lots as a result of seed hulling. He concluded that this was due to the fact that most of the inoculum was carried on or in the hulls and, therefore, was removed with them in the process of hulling. It is interesting to note that Gage's results were obtained under greenhouse conditions very favorable to smut infection and that Johnston's results were obtained under relatively unfavorable field conditions. It, therefore, seemed possible that environmental conditions might be related to the influence of seed hulling on loose smut in naturally inoculated oats. It was evident also that if it were found that the hulls have an important influence on the enclosed inoculum of florally inoculated seed, this knowledge would aid in evaluating the reliability of results from various artificial methods of seed inoculation in which the inoculum is not enclosed by the hulls. As noted later, some apparently valid objections to the methods of applying dry spores to nonhulled or to hulled seed previously have been raised on other bases.

MATERIALS AND METHODS

From a collection of naturally inoculated seed lots from the crop of 1928, 10 lots were selected that were infested largely or entirely with the loose smut, as shown in a greenhouse planting in the winter of 1928-29. The 10 lots were collected from 5 States and represented 5 different varieties, strains or mixtures. Sowings were made in the field and greenhouse at the

Arlington Experiment Farm, Rosslyn, Virginia, and in an irrigated field at Aberdeen, Idaho.

In all of the tests the hulls were removed within a period of 3 weeks or less before sowing. In the field experiment at the Arlington farm, 250 non-hulled and 250 hulled seeds of each of the 10 lots were sown in adjacent rod rows 1 ft. apart. The field planting at Aberdeen was similar except that only 7 lots were used and the rows were 7 feet long, 100 seeds to the row. In the greenhouse experiment at the Arlington farm, 100 nonhulled and 100 hulled seeds of each of the 10 lots were sown in an alternating series of 8 42-inch rows, 25 seeds per row.

RESULTS

The results of the experiment are presented in table 1.

Table 1 shows that seed hulling consistently produced some reduction in the amount of smut. However, the degree of reduction varied widely, both within individual lots grown under different conditions and between different lots grown under similar conditions. The data, therefore, have little value in determining whether the inoculum in the hulls or pericarp is accountable for most of the smut in oats caused by *Ustilago avenae*.

Within individual lots grown under the different field and greenhouse conditions, the differences in smut reduction due to seed hulling were particularly striking, ranging from as little as 9.4 per cent in the case of lot 1 to as much as 88.1 per cent in lot 3. In the latter the percentage reductions in smutted plants resulting from hulling were 2.1 in the greenhouse at Rosslyn, 24.3 in the field at Aberdeen, and 90.2 in the field at Rosslyn. The greenhouse data indicate that the effective inoculum resided largely in the pericarp of the caryopsis, as removal of the hulls produced only a slight reduction in the percentage of smutted plants. The Rosslyn field data, however, indicate that the effective inoculum resided largely in the hulls. Similar inconsistencies are obtained in comparing the different lots grown under similar conditions. Hulling effected reductions in the percentage of smutted plants ranging from 24.3 to 73.2 in the field at Aberdeen, from 72 to 100 in the field at Rosslyn, and from 2.1 to 91.1 in the greenhouse at Rosslyn.

Further examination of table 1 shows that, in general, relatively unfavorable conditions for smuttedness were more acutely reflected in plants from hulled seed than in plants from nonhulled seed. In the ten lots grown at Rosslyn, the average percentages of smutted plants from nonhulled seed were 13.8 in the field and 43.6 in the greenhouse. Under the relatively unfavorable field conditions, the reduction in smutted plants due to seed hulling was 89.9 per cent. In the greenhouse the reduction was only 48.2 per cent. In the 7 lots grown in the field, both at Aberdeen and Rosslyn, the average, 14.8 per cent, of smutted plants from nonhulled seed at Rosslyn

TABLE 1.—*Influence of hulling the caryopsis on loose smut in oats. Plants grown from naturally inoculated seed sown in the field at Aberdeen, Idaho, and at Rosslyn, Virginia (Arlington Experiment Farm), in the spring of 1929 and in the greenhouse at Rosslyn in the winter of 1929-30. Seven lots were sown at Aberdeen and 10 lots in the field and greenhouse at Rosslyn*

Seedlot number, variety, and source	Location of experiment	Seed nonhulled (n) or hulled (h)	Plants matured			Reduction in smutted plants due to seed hulling
			Total	Smutted		
			Number	Number	Per cent	Per cent
1 Green Russian from Iowa	Field, Rosslyn, Va.	(n)	197	13	6.6	90.9
		(h)	163	1	0.6	
	Greenhouse, Rosslyn, Va.	(n)	97	12	12.4	81.5
		(h)	87	2	2.3	
2 Green Russian from Iowa	Field, Aberdeen, Ida.	(n)	73	9	12.3	30.1
		(h)	81	7	8.6	
	Field, Rosslyn, Va.	(n)	215	25	11.6	85.3
		(h)	181	3	1.7	
3 Green Russian from Iowa	Greenhouse, Rosslyn, Va.	(n)	99	48	48.5	21.0
		(h)	94	36	38.3	
	Field, Aberdeen, Ida.	(n)	78	9	11.5	24.3
		(h)	69	6	8.7	
4 Frazier from Texas	Field, Rosslyn, Va.	(n)	195	26	13.3	90.2
		(h)	158	2	1.3	
	Greenhouse, Rosslyn, Va.	(n)	98	42	42.9	2.1
		(h)	88	37	42.0	
5 A mixture from New Jersey	Field, Aberdeen, Ida.	(n)	85	13	15.3	73.2
		(h)	73	3	4.1	
	Field, Rosslyn, Va.	(n)	231	27	11.7	100.0
		(h)	205	0	0	
6 Kanota from Kansas	Greenhouse, Rosslyn, Va.	(n)	89	41	46.1	91.1
		(h)	73	3	4.1	
	Field, Aberdeen, Ida.	(n)	82	19	23.2	57.3
		(h)	71	7	9.9	
7 Kanota from Kansas	Field, Rosslyn, Va.	(n)	202	49	24.3	72.0
		(h)	207	14	6.8	
	Greenhouse, Rosslyn, Va.	(n)	94	61	64.9	52.9
		(h)	85	26	30.6	
8 Kanota from Kansas	Field, Aberdeen, Ida.	(n)	92	8	8.7	27.6
		(h)	64	4	6.3	
	Field, Rosslyn, Va.	(n)	247	27	10.9	96.3
		(h)	223	1	0.4	
9 Kanota from Kansas	Greenhouse, Rosslyn, Va.	(n)	100	21	21.0	47.6
		(h)	100	11	11.0	
	Field, Aberdeen, Ida.	(n)	88	18	20.5	48.8
		(h)	76	8	10.5	
10 Kanota from Kansas	Field, Rosslyn, Va.	(n)	242	43	17.8	100.0
		(h)	237	0	0	
	Greenhouse, Rosslyn, Va.	(n)	75	39	52.0	72.1
		(h)	62	9	14.5	

TABLE 1.—(Continued)

Seedlot number, variety, and source	Location of experiment	Seed nonhulled (n) or hulled (h)	Plants matured			Reduction in smutted plants due to seed hulling
			Total	Smutted		
			<i>Number</i>	<i>Number</i>	<i>Per cent</i>	<i>Per cent</i>
8 Kanota from Kansas	Field, Rosslyn,	(n)	225	16	7.1	93.0
	Va.	(h)	211	1	0.5	
	Greenhouse, Rosslyn, Va.	(n)	98	24	24.5	74.3
		(h)	80	5	6.3	
9 Swedish Select from Wisconsin	Field, Aberdeen,	(n)	61	9	14.8	34.4
	Ida.	(h)	72	7	9.7	
	Field, Rosslyn,	(n)	220	32	14.5	78.6
	Va.	(h)	225	7	3.1	
	Greenhouse, Rosslyn, Va.	(n)	84	74	88.1	33.3
		(h)	97	57	58.8	
10 Kanota from Kansas	Field, Rosslyn,	(n)	226	46	20.4	100.0
	Va.	(h)	209	0	0	
	Greenhouse, Rosslyn, Va.	(n)	90	41	45.6	86.2
		(h)	79	5	6.3	
Lots 2, 3, 4, 5, 6, 7, and 9	Field, Aberdeen,	(n)	559	85	15.2	45.4
	Ida.	(h)	506	42	8.3	
	Field, Rosslyn,	(n)	1552	229	14.8	87.2
	Va.	(h)	1436	27	1.9	
	Greenhouse, Rosslyn, Va.	(n)	639	326	51.0	41.4
		(h)	599	179	29.9	
All lots	Field, Rosslyn,	(n)	2200	304	13.8	89.9
	Va.	(h)	2019	29	1.4	
	Greenhouse, Rosslyn, Va.	(n)	924	403	43.6	48.2
		(h)	845	191	22.6	

was only slightly less than the 15.2 per cent at Aberdeen. The average reduction in smut resulting from seed hulling at Rosslyn (87.2 per cent) was, however, almost twice that at Aberdeen (45.4 per cent). Finally, comparison of data from the 7 lots grown in the field at Aberdeen and the greenhouse at Rosslyn shows similar results, but in much less striking degree. The average percentages of smut from nonhulled seed were 15.2 at Aberdeen and 51.0 at Rosslyn. The average reductions in smut resulting from seed hulling were 45.4 per cent at Aberdeen and 41.4 per cent at Rosslyn. It is interesting also to note that the divergent results of Gage (3) and Johnston (6) referred to above may be explained on the basis that conditions unfavorable to smut are more pronounced in plants from hulled seed than in those from nonhulled seed. Gage obtained a reduction in smutted plants of only 8.2 per cent from hulled seed under greenhouse conditions that resulted in 67.0 per cent of smutted plants from the nonhulled seed. Johnston, however, obtained reductions up to 100 per cent from seed hulling, under field condi-

tions relatively unfavorable to the development of smut in plants from the nonhulled seed.

The data of table 1 show further that hulled seed of all of the lots produced some smutted plants; evidence that at least a portion of the inoculum resided in the caryopses of these lots.

With reference to the number of plants obtained from hulled and non-hulled seed, the data of table 1 give further support to previous investigations (9) in showing that, in general, seed hulling results in a reduced stand.

DISCUSSION

From the data presented, it is evident that the removal of hulls from naturally inoculated seed oats involves factors in addition to reduction in inoculum load that the hulls may carry. Considering the nature of the tightly appressed hulls, it seems possible that they may serve also as buffers in protecting the inoculum in the pericarp against dessication, flooding, sudden changes in temperature, possible antagonistic organisms and other soil factors that inhibit or prevent infection. As noted, the apparent influence of the hulls in this respect varied widely under different environmental conditions. Doubtless the possible interplay of different soil factors covers a wide range of conditions that might variously influence both the protective action of the hulls and the physiologic relations of the various combinations of host varieties and physiologic forms of the pathogen in the different seed lots.

In a consideration of methods of artificially inoculating seed oats with loose smut, it seems evident, therefore, that the position of inoculum with reference to protection by the hulls may prove to be an important factor in the approach to results comparable to those in nature. This protection is not obtained by coating the surface of nonhulled seed with dry smut spores. Furthermore, as shown by Johnston (6), this method also may enable susceptible varieties to escape infection through the interference of the hulls. Application of dry spores to hulled seed eliminates the latter factor and generally results in high percentages of smut, as shown by Stanton *et al.* (9). Hulling, however, is a slow and tedious operation. Zade and Arland (13) report that it required 32 8-hour days to hull and inoculate one kilogram (2.2 lbs.) of oats. These investigators also observed that under natural conditions and with the "evacuation" method of inoculation, plants from the inner seeds of the spikelets are more susceptible than those from the outer seeds. The application of a heavy spore load to the hulled seed, however, resulted in an attack so severe as to obliterate this natural difference. A further complication is introduced by the fact shown by Stanton *et al.* (9) that hulling not only reduced seedling emergence but the extent of reduction varies considerably in different varieties. The writer's recent observa-

tions on different lots of oats have shown a notable difference in the susceptibility of nonhulled and hulled seed to mold attack. The shrivelled, hulled seeds attached to week-old seedlings dug from the soil, were thickly covered with blue, black, and pink molds. The comparable nonhulled seeds were bright and macroscopically mold-free. In view of this fact, the mold attack rather than injury from the process of hulling may be chiefly responsible for the reductions in seedling emergence that usually accompanies hulling. As shown by Stanton *et al.* (9) these reductions occur even when the seed is not inoculated with smut. The likely influence of this unnatural prevalence of mold on the smut inoculum of hulled seed doubtless raises a further question as to the desirability of using hulled oats.

In the light of the results here recorded it should be noted, moreover, that the method of applying spores to hulled seed fails to include not only the influence of the protection of inoculum by the hulls but also the variable extent of this protection occasioned by the fact that the length, thickness, and adhesiveness of the hulls varies in different varieties.

The "evacuation" inoculation method described by Zade (12) and Haarring (4, 5) apparently is more satisfactory in expediting results comparable to those in nature. In this method, seed is placed in a spore suspension and the spores are forced beneath the hulls by the release of a vacuum. The moist seed is then stored for 20 hours to promote spore germination and the spread of subsurface inoculum. This method, however, would be difficult to apply in physiologic-form studies, as it is necessary to inoculate many small lots of seed with different collections of smut. Doubtless further experimentation would be desirable to determine the comparability of results from various artificial methods of seed inoculation and from the natural method, under widely different conditions of seeding.

SUMMARY AND CONCLUSIONS

In the usual artificial methods of inoculating seed oats with loose smut, dry spores are applied to the surface of nonhulled or hulled seed. The inoculum, therefore, comes into direct contact with the soil, whereas the effective inoculum in naturally inoculated seed resides in the superficial seed structures enclosed and protected by the siliceous layers of the hulls.

To determine the significance of the protection afforded by the hulls in seedling infection and on subsequent smut in oats, nonhulled and hulled seed of 10 seed lots, naturally inoculated with loose smut, was sown in the field and greenhouse at Rosslyn, Va. (Arlington Experiment Farm). Seven of the lots also were sown in the field at Aberdeen, Idaho, and grown under irrigation. This procedure also permitted a further study of the moot question whether inoculum in the hulls or pericarp is accountable for most of the

seedling invasion and loose smut in oats grown from naturally inoculated seed.

The results showed that hulling is not a reliable basis on which to determine whether inoculum in the hulls or pericarp is accountable for most of the loose smut in oats. The different conditions in the 3 locations of the experiment so markedly influenced the range of reduction in smut due to hulling that inoculum either in the hulls or pericarp could be held accountable for most of the smut. Within individual lots the percentages of reduction in smut in plants grown from hulled seed varied as widely as from 2.1 to 90.2 in the 3 locations of the experiment. In the different lots grown under similar conditions wide variations in smut reduction from hulling also occurred.

In general, relatively unfavorable conditions for smut were more acutely reflected in plants from hulled seed than in plants from nonhulled seed.

The inconsistent reductions in smut resulting from hulling indicate that hulling involves factors in addition to reduction in the load of inoculum carried by the hulls. Apparently the tightly adherent hulls also may serve more or less effectively as buffers in protecting inoculum in the pericarp against various soil influences inimical to infection. In the present experiments the extent of this influence varied widely under different conditions.

Hulled seed of all of the 10 seed lots produced some smutted plants; evidence that at least a portion of the loose-smut inoculum resided in the caryopses of these lots.

In a discussion of the various artificial methods of inoculating seed oats with loose smut, it is noted that present methods are laborious and/or not applicable to all types of investigation and/or that they fail to involve the influence of the seed hulls and introduce unnatural and variable factors that may mask the true status of resistance or susceptibility as it occurs in nature. Further experimentation is desirable to determine the comparability of results from various artificial methods of seed inoculation and from the natural method, under widely different conditions of seeding.

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COMPARATIVE STUDIES OF SOME EUROPEAN AND AMERICAN POTATO VIROSES¹

T. P. DYKSTRA

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Viroses affecting potatoes have been described in Europe and America. Since the potato varieties in Europe differ from those in America and the symptoms of potato viroses may vary considerably, depending upon the variety, it is impossible to determine the identity of the viruses found on the two continents unless the diseases caused by them are studied on the same varieties under similar conditions.

Until the identity of the viroses affecting the potato in Europe and America is determined, it is not possible to apply the extensive knowledge gained in one continent to the solution of related problems of the other.

In order to study foreign and American viroses on European as well as American varieties, several virus-diseased and healthy tubers of different varieties were secured from England through the courtesy of Dr. R. N. Salaman. Since healthy tubers of European varieties are generally free from the X-virus (latent mosaic universally present in so-called "healthy" commercial American varieties), it was felt desirable for comparison of the viroses to remove the X-component from the American virus complexes. This was readily accomplished by grafting scions of virus-diseased potatoes to the U.S.D.A. Seedling No. 41956, which is resistant to this latent virus (8). Tubers of this seedling were kindly supplied by E. S. Schultz, of the Bureau of Plant Industry, United States Department of Agriculture.

By this procedure, it was possible to secure leaf-rolling mosaic, crinkle mosaic, mild mosaic, and rugose mosaic free from the X component. These virus components, with the possible exception of leaf-rolling mosaic also were secured free from the X virus by transferring the diseases to tobacco by means of aphids, which do not transfer the latent potato mosaic.

The European viroses have been studied in comparison with American material under the same conditions on different American and European varieties, and in many cases on plants that came from the same tuber unit. Although the work is not nearly completed, sufficient progress has been made to warrant drawing certain conclusions. It is believed opportune to present this preliminary note.

The studies were started in the greenhouse at Corvallis, Oregon, in the

¹ This paper is based upon investigations carried on as a cooperative project between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, and the Oregon Experiment Station at Corvallis, Oregon.

fall of 1933. It was found that, with the exception of the Y virus, the grafting method of transferring the European viroses was far more successful than the leaf-mutilation method. Stem grafts of diseased scions were made to healthy potted plants, which were then kept in a moist chamber for about 10 days. Then they were removed and kept in the greenhouse for further observations, according to the method recommended by Salaman and Le Pelley (7). When this procedure was followed, about 80 per cent of the grafts were successful.

After the plants had matured, the tubers were harvested, stored in paper bags, and planted the following spring under muslin-covered insect-proof cages in the field. This procedure makes it possible to secure current-season, and tuber-perpetuated symptoms within a year.

The general conclusions reached are summarized in the following paragraphs:

Y Virus and Vein-banding Virus. Smith (9) refers to potato virus Y as one of the commonest and most destructive of potato viruses in England. He states that it is common in American tobacco fields, where it is known as the vein-banding virus. The American vein-banding virus is the aphid-transmitted component of rugose mosaic. Koch (1). This virus has been studied by the writer in comparison with the Y virus on tobacco and on different American and European potato varieties.

Both of these viroses produce on tobacco a banding of the veins, but the symptoms produced by the Y virus are very striking and distinct, whereas those produced by the vein-banding virus are very mild in comparison. Both of these viroses in conjunction with virus X produce spot necrosis on tobacco.

The current-season symptoms of the Y virus on Irish Cobbler, White Rose, Burbank, Bliss Triumph, Green Mountain, and Earliest of All, which also harbor X virus, are manifested by necrotic leaf spots including the veins and the tissue immediately surrounding them. Nearly every leaf on the plant may eventually drop, and remain hanging on the main stem by a thread of tissue, leaving only a tuft of leaves at the tip of the plant.

Plants from tubers of plants inoculated with Y virus appear at first as if infected with rugose mosaic, except that the necrosis of the veins instead of being confined to the lower leaves, is found on almost every leaf. While the plant is still immature, the lower leaves, at first green, gradually turn yellow and drop, but remain hanging on the stem by a thread of tissue (fig. 1).

In the variety Bliss Triumph the petioles develop necrotic spots and stripes of streak are found on the stem. Finally, all of the lower leaves dry down and hang on the main stem. At this stage of development the disease appears to be quite different from rugose mosaic.

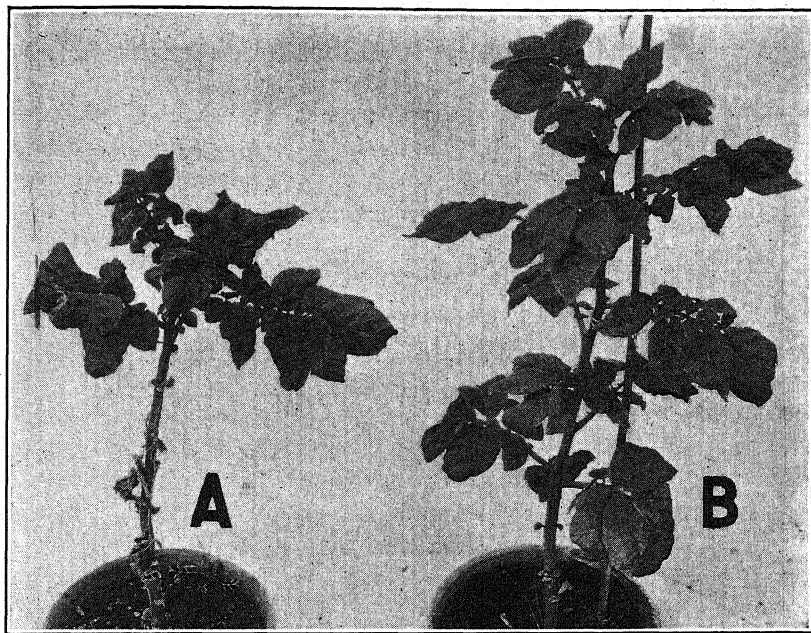


FIG. 1. Tuber-perpetuated symptoms of viroses on Bliss Triumph in the greenhouse. A. Y virus necrosis of the veins is evident on practically every leaf. Note the severe leaf drop. B. Vein-banding virus. Necrosis of the veins is evident on the lower leaves only. Note the absence of leaf drop.

When the vein-banding virus is inoculated into potato plants containing the X virus, current-season symptoms of rugose mosaic develop. It was found that under greenhouse conditions the leaves did not always develop a necrotic spotting, as a result of inoculation of the above-named varieties with the vein-banding virus. In some cases a diffused mottling, typical of rugose mosaic, developed without any noticeable necrosis.

Plants from tubers of plants inoculated with vein-banding virus showed typical rugose-mosaic symptoms, evidenced by a rugosity of all the leaves and a necrosis of the veins of the lower leaves. In no case was any leaf dropping observed on tuber-perpetuated rugose mosaic plants of the different varieties tested. In these cases, necrosis was not evident in the petioles, nor was streaking of the stem observed in infected plants of the variety Bliss Triumph.

In the English variety President, the symptoms of the second generation of the Y virus manifest considerable leaf drop and considerable necrosis of the veins in addition to crinkling and diffused mottling of the leaves.

The tuber-perpetuated symptoms of the vein-banding virus on President differ from the Y virus in that it fails to cause an appreciable necrosis of

the veins or leaf drop, but results only in a diffused mottling of the leaves. The symptoms of these two viroses in this variety have been observed on more than 100 plants.

Both of these viroses cause on Arran Victory diffused mottling without any necrosis or leaf drop. The vein-banding virus causes only a diffused mottling on Epicure, whereas the Y virus caused a diffused mottling and a slight necrosis of the veins of the lower leaves.

In order to determine whether or not the Y virus has any effect upon potato plants infected with rugose mosaic the following experiment was started in the spring of 1935. Each of 6 tubers of the variety Green Mountain and 6 tubers of Bliss Triumph infected with rugose mosaic were cut into 3 pieces. Each seed piece was planted in a 6-in. pot in the greenhouse. When the plants were about 3 or 4 inches high, those derived from 1 seed-piece of each tuber were inoculated with the Y virus by means of the rubbing method. Plants from another seedpiece of each tuber were inoculated with the vein-banding virus, and the other seedpieces were not inoculated and served as controls.

These plants were kept under observation for more than 2 months after inoculation, but none of the plants developed symptoms different from the controls, which showed the typical tuber-perpetuated rugose-mosaic symptoms.

It appeared as if the rugose-mosaic virus had an inhibiting effect upon the expression of the Y virus and prevented the development of the typical Y-virus symptoms. The tubers from the inoculated plants were saved and will be planted later to determine whether the introduction of the Y virus or additional vein-banding virus into rugose-mosaic-infected potato plants has any effect on the symptoms in the progeny.

Further studies with these 2 viroses are in progress, but the results secured to date have proven conclusively that they belong to the vein-banding group but are not identical. The effect of the Y virus on potato plants is more severe than that of the vein-banding virus.

Paracrinkle. This disease was first described by Salaman and Le Pelley (7). Murphy and McKay (2) have suggested that this virus may have affinities with leaf-rolling mosaic. We have studied this virus in comparison with leaf-rolling mosaic on the American varieties, Burbank, White Rose, Earliest of All, and Bliss Triumph, and failed to find any resemblance in the symptoms of these two diseases. Instead of diffused mottling and a rolling of the leaves, which are the characteristic symptoms of leaf-rolling mosaic, paracrinkle developed large mosaic-like blotches on the foliage, and failed to produce any rolling of the leaves.

Leaf-rolling mosaic, free from X virus, as well as the complex leaf-rolling

mosaic and the X virus, have been transferred to Arran Victory. The symptoms of this disease on this variety are characterized by a diffused mottling and rolling of the leaves in contrast to the blotchy mottling and puckering of the leaves caused by paracrinkle.

In the variety Burbank, pin-point-like necrotic spots developed on the leaves in addition to the mottling when infected tuber-perpetuated plants were grown under cages in the field. (Fig. 2.) These necrotic spots, how-

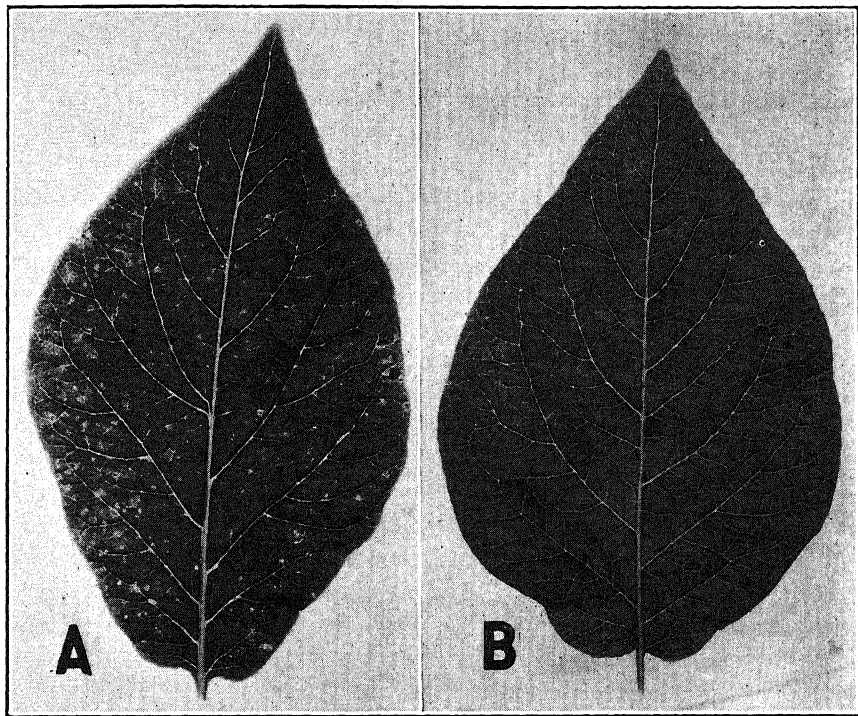


FIG. 2. Tuber-perpetuated paracrinkle symptoms on Burbank. A. On plants growing under cages in the field. Note pin-point-like necrotic spots on leaves. On the print they resemble thrips injury, but these insects were not present in the cage. B. In the greenhouse. Note the large irregularly shaped mosaic-like blotches.

ever, were not evident on the progeny from these plants when planted in the greenhouse. Paracrinkle appears to be entirely different from any of our American diseases.

Crinkle A. This disease is well known in Europe and has been described as crinkle by Murphy, Quanjér, and Salaman (2, 3, 4, 6). The last of whom refers to it as crinkle A to distinguish it from paracrinkle.

It is composed of at least one virus component in addition to X. That

the X virus is the same as the American latent mosaic (present in commercial "healthy" potatoes) was indicated when juice transfer of this virus to Jimson weed resulted in the characteristic mottling of the leaves. By grafting scions of infected President to seedling No. 41956 (resistant to the latent virus), the X virus was eliminated and the other component was retained. This component has been studied on tobacco plants in comparison with the viruses from American mild mosaic and crinkle mosaic from which the latent mosaic component had been removed. There is no observable consistent difference in the symptoms produced by these three viroses on tobacco plants. Instead of vein-banding, a mottling develops which resembles the mild mottling sometimes produced by the X virus. It was found that it can be readily distinguished from this virus, however, by inoculating infected plants with tobacco mosaic. The tobacco plants infected with the X virus upon addition of tobacco mosaic will invariably develop a spot necrosis on the leaves, whereas the plants infected with the other viruses will develop only tobacco-mosaic symptoms in addition to the mottling due to the particular potato-mosaic virus present.

These viruses are readily transmitted to tobacco, and 100 per cent infection was invariably secured when a small amount of carborundum dust was added to the extracted juice from the leaves of an infected plant, and a piece of absorbent cotton rolled into the end of a swab stick was dipped into the juice and rubbed lightly on the tobacco leaves (5).

These three viroses, namely, mild mosaic, crinkle mosaic, and Crinkle A, free from the latent virus, have been transferred from tobacco to Bliss Triumph potato plants by juice transfers and also by grafting, and have resulted in the development of typical symptoms characteristic of each disease in this variety.

Studies on the identity of crinkle A are not yet completed, but the results secured indicate its resemblance to those of mild mosaic, and crinkle mosaic, yet it differs from them. The most striking difference in symptoms among these three viroses was found on the X resistant seedling. On this host the crinkle-A virus fails to produce any appreciable leaf crinkle, and, while the mottling is more uniformly spread over the leaf, the mottled areas are not so chlorotic as those produced by the other two viroses (Fig. 3). Crinkle mosaic resembles mild mosaic on this host, except that the former causes considerably more crinkling of the foliage. These three diseases also have been studied on Bliss Triumph, Green Mountain, Earliest of All, and Burbank, and, although there is a difference in symptoms produced on these varieties, it is not so pronounced as in the seedling. Crinkle A resembled mild mosaic more than crinkle mosaic. Of three diseases, crinkle A produced the least crinkling of the leaves.

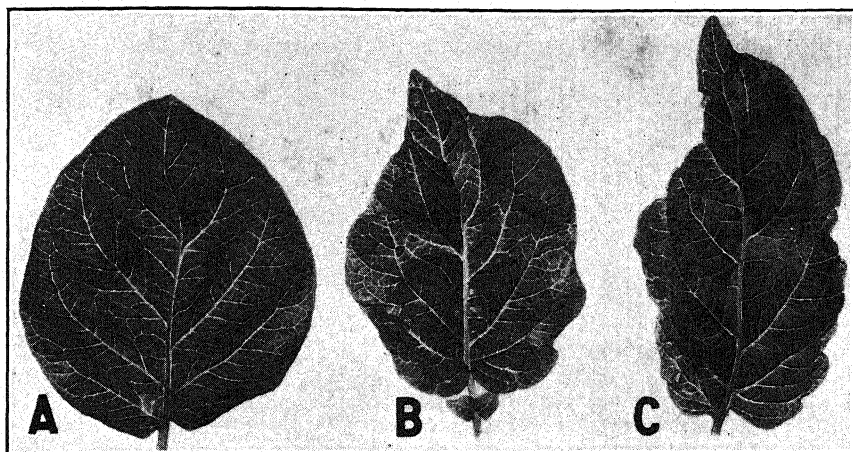


FIG. 3. Leaflets of X resistant U. S. D. A. seedling No. 41956 showing tuber-perpetuated symptoms of the following diseases: A. Crinkle A. B. Mild-mosaic. C. Crinkle mosaic. Note the variation in types of mottling and degrees of crinkling produced in these three different mosaics.

Murphy and McKay (2) also state that crinkle A is closely related to the American mild mosaic.

Top necrosis Di Vernon. This virus has been described by Bowden (according to Smith) (10, p. 317) who refers to the component causing top necrosis in certain potato varieties as virus C. In addition to this the X component also is present.

In the fall of 1933 a few grafts of scions from infected Di Vernon plants were made to some American potato varieties, but current season symptoms failed to develop. When the progeny from these plants were planted under cages, only two plants from one hill lot of Bliss Triumph developed symptoms, which were characterized by extreme dwarfing of the plant and a necrosis of the veins of most of the leaves.

In the fall of 1934 several plants of the variety Burbank, Bliss Triumph, Irish Cobbler, and Earliest of All were grafted with scions from infected Di Vernon plants. In nearly all cases current-season symptoms developed that were characterized in these varieties by a severe top necrosis, consisting of a streaking of the stem and petioles, and by numerous small circular necrotic spots on the foliage (fig. 4).

The second generation symptoms of this disease on these varieties as observed on plants growing under cages in 1935, were manifested by a slight mottling of the foliage, without any noticeable dwarfing and in many cases without any indications of necrosis. In a few plants of Irish Cobbler circular necrotic spots were found on the lower leaves.

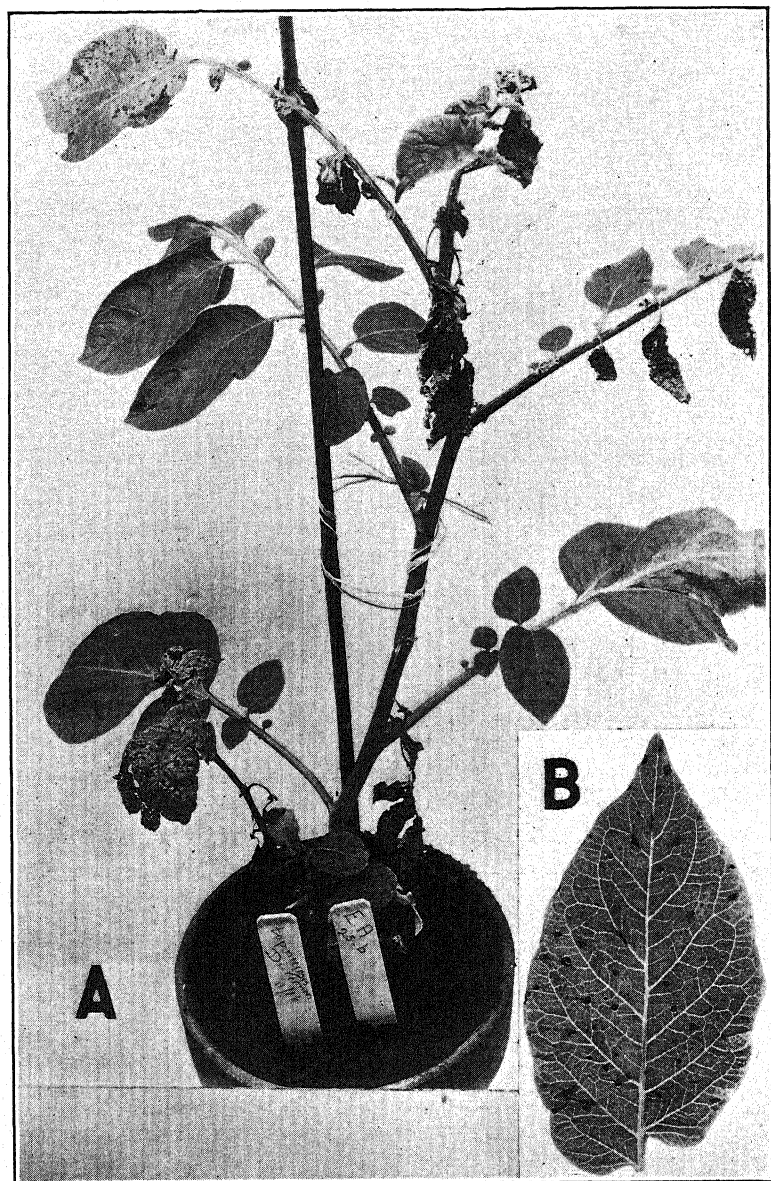


FIG. 4. Current-season symptoms of top necrosis produced by virus C. A. On Earliest of All. Note necrotic spotting on the foliage and petiole. B. On Irish Cobbler. Note necrotic spots.

The fact that in the one positive case of 1934 current-season symptoms had failed to develop, may account for the severe effect of this disease in the second generation. The writer has observed that in some cases when rugose-mosaic infection took place late in the season and current-season symptoms failed to develop, the second generation symptoms the following year closely resembled current-season infection.

The X component has been removed from this virus complex by grafting scions from plants affected with top necrosis Di Vernon to resistant seedling No. 41956. This C virus has successfully been transferred from this seedling to President and Majestic. The effect of this virus on plants free from the X virus has not yet been extensively studied.

Virus C does not correspond to any of the known potato viruses found in America.

SUMMARY

Comparisons of some European and American potato viroses were made on a number of potato varieties from both continents. The X virus was found to be similar to the so-called latent virus of "healthy" American commercial potatoes. The Y virus, and the vein-banding virus (rugose mosaic complex from which the X component has been removed) produce a banding of the veins on tobacco leaves. These two viroses belong to the same group but are not identical. Whereas the tuber-perpetuated vein-banding symptoms are manifested on most American varieties containing the X virus by a necrosis of the veins of the lower leaves, without leaf drop, American varieties infected with tuber-perpetuated Y virus showed not only a necrosis of the veins on practically every leaf of the plant but also considerable leaf drop. This virus caused a necrosis of the petioles and streaking in the stem of Bliss Triumph.

Paracrinkle does not resemble leaf-rolling mosaic or any other known American potato virosis. It produces large, irregular-shape mosaic-like blotches on the foliage of American varieties. Under cages, in the field, pin-point-like necrotic spots, in addition to a mottling, developed in the leaves of infected Burbank plants.

Crinkle A is not identical with rugose mosaic. It resembles mild mosaic, but the mottled pattern is somewhat different and the crinkling of the leaves is less severe than that caused by mild mosaic on the same varieties.

Virus C produced a severe top necrosis as current season symptoms on every one of the American potato varieties tested. This virus does not resemble any of the known viruses affecting potatoes in this country.

OREGON STATE COLLEGE,
CORVALLIS, OREGON.

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PHYTOPATHOLOGICAL NOTES

An Apparent Natural Transfer of the Bulb or Stem Nematode from Clover to the Strawberry Plant.—An instance of what appears to be a natural transfer of the bulb or stem nematode, *Anguillulina dipsaci*, from red clover to Marshall strawberries has been observed in the Pacific Northwest.

The strawberry plants were grown on part of a field formerly in red clover that had a known infestation by the bulb or stem nematode for the last several years. During 1933 the farmer plowed a portion of this field and planted the newly plowed area to Marshall strawberries (Fig. 1). In

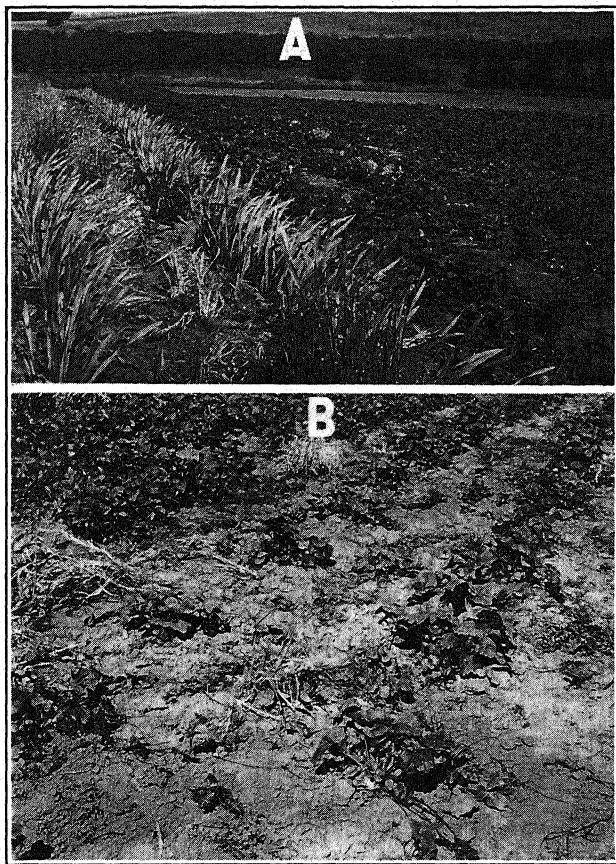


FIG. 1. A. General view of strawberry field showing two rows of uninfested narcissus plants on left. Blank area at back is location where strawberry runners failed to grow, due to *A. dipsaci*. B. Closeup of infested area in strawberry field shown at left. Note irregular stand and distorted plant in foreground.

the early spring of 1935, 4 acres more of the infested field were plowed and planted with runners from the above mentioned strawberry plants. When these young plants failed to grow properly, the farmer called his county horticultural inspector who submitted clover plants from the original clover field and the mother strawberry plants and runners to the present writer for

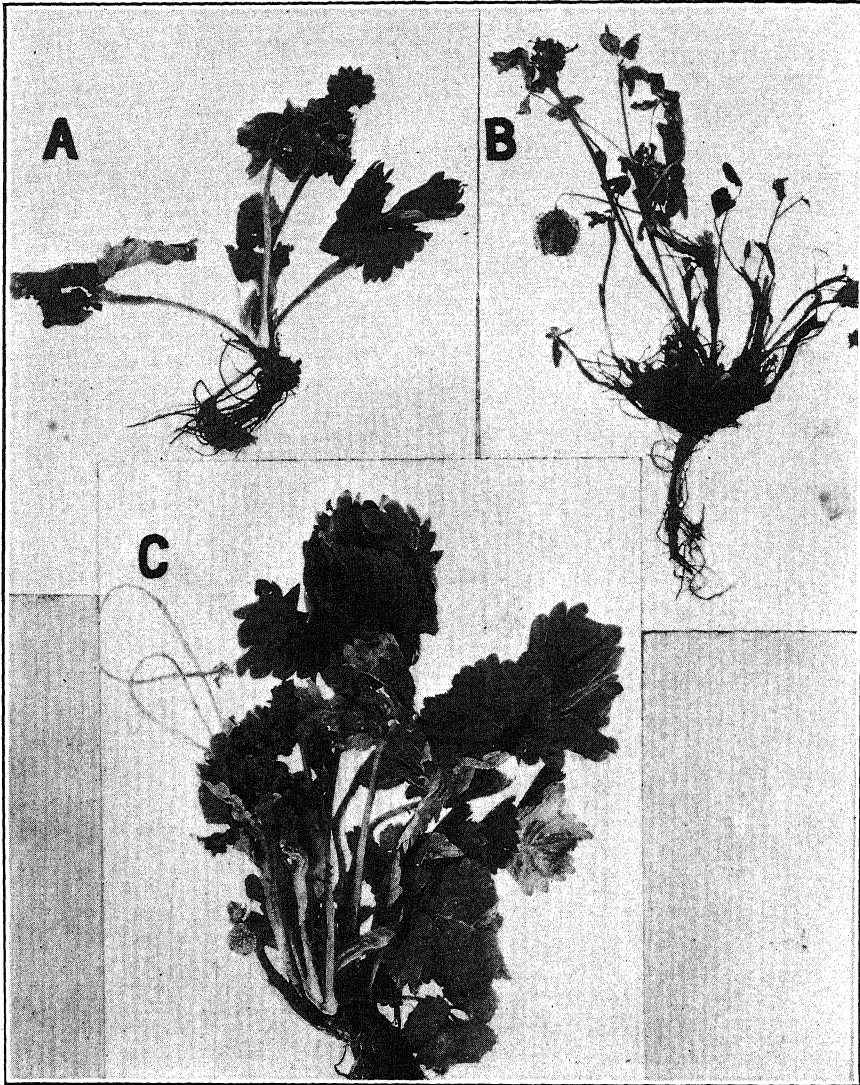


FIG. 2. A. Runner showing swollen stems and leaf distortion caused by *A. dipsaci*. B. Clover plant nearly killed by infestation of *A. dipsaci*. C. Mother plant showing same symptoms as runner.

an examination as to the presence of a nemic disease. All these plants exhibited marked nemic-disease symptoms (Fig. 2), and upon microscopic examination, showed large numbers of *Anguillulina dipsaci* in all stages of development.

From the following circumstantial evidence it seems probable that in the present case the red-clover population of *Anguillulina dipsaci* has transferred, under natural conditions, to Marshall strawberries:

1. A check of the source of supply of the mother strawberry plants failed to show any nematode infestation or history.
2. Other strawberry growers, who secured their planting stock from the same place at the same time as did the grower concerned herein, have no nemic infestation in their fields.
3. The field on which the strawberry plants were grown had never before been planted to strawberries.

Infested plant material has been secured from these fields, so that experimental evidence also may be furnished.

In view of the importance of the host specificity problem in the study of plant parasitic nematodes and their control, observations like the present one are of so much value that they should be recorded.—WILBUR D. COURTNEY, Sumner, Washington.

Greenhouse Wires and Pipes Galvanized with Zinc React with Sulphur Dioxide to Form Soluble Zinc Salts.—In the summer of 1935 sulphur dioxide fumigation, along with formaldehyde soil sterilization, was recommended to help clean up several greenhouse diseases, but later in the fall severe injury occurred to a crop of tomatoes. The outbreak of the injury always was correlated with the dripping of condensed moisture or rains from wires and pipes. Yellowish white to white crystals were observed on all zinc-galvanized surfaces. In the presence of water these crystals dissolved slowly and the solution dripped on the plants below. Wherever the solution touched the tomato plants, black to blackish brown lesions were produced. In many cases the plants were severely injured. This same injury was reported in 1933 by Read and Orchard¹ on chrysanthemums under somewhat similar conditions in England. These workers observed that zinc sulphate was formed on galvanized wires and pipes as well as surfaces that were painted with paints containing "zinc white" (zinc oxide) as their base, if sulphur was burned in a humid atmosphere.

The problem in Illinois deals with wires and pipes galvanized with metallic zinc. If fumigation is done in a humid atmosphere the moist sul-

¹ Read, W. H., and Orchard, O. B. Plant injury following the burning of sulfur in glasshouses. Experimental and Research Station, Turner's Hill, Cheshunt, Herts. Ann. Rpt. 19: 98-108, 1933 (4).

phur dioxide can be expected to react with the zinc on the pipes, wherever moisture has condensed, to form zinc thiosulphate,² a soluble greenish yellow solid with the formula ZnS_2O_3 . Under greenhouse conditions it is very probable that the zinc thiosulphate would oxidize to zinc sulphate. Both of the salts are soluble in water and capable of injuring plants if a too concentrated solution touches them.—K. J. KADOW, W. A. RUTH and H. W. ANDERSON, University of Illinois, Urbana, Illinois.

² Roscoe, H. E., and Schorlemmer, C. Treatise on Chemistry 2: 6th Edition, 1923. MacMillan.

PHYTOPATHOLOGICAL SUMMER MEETING

IOWA STATE COLLEGE, AMES, IOWA

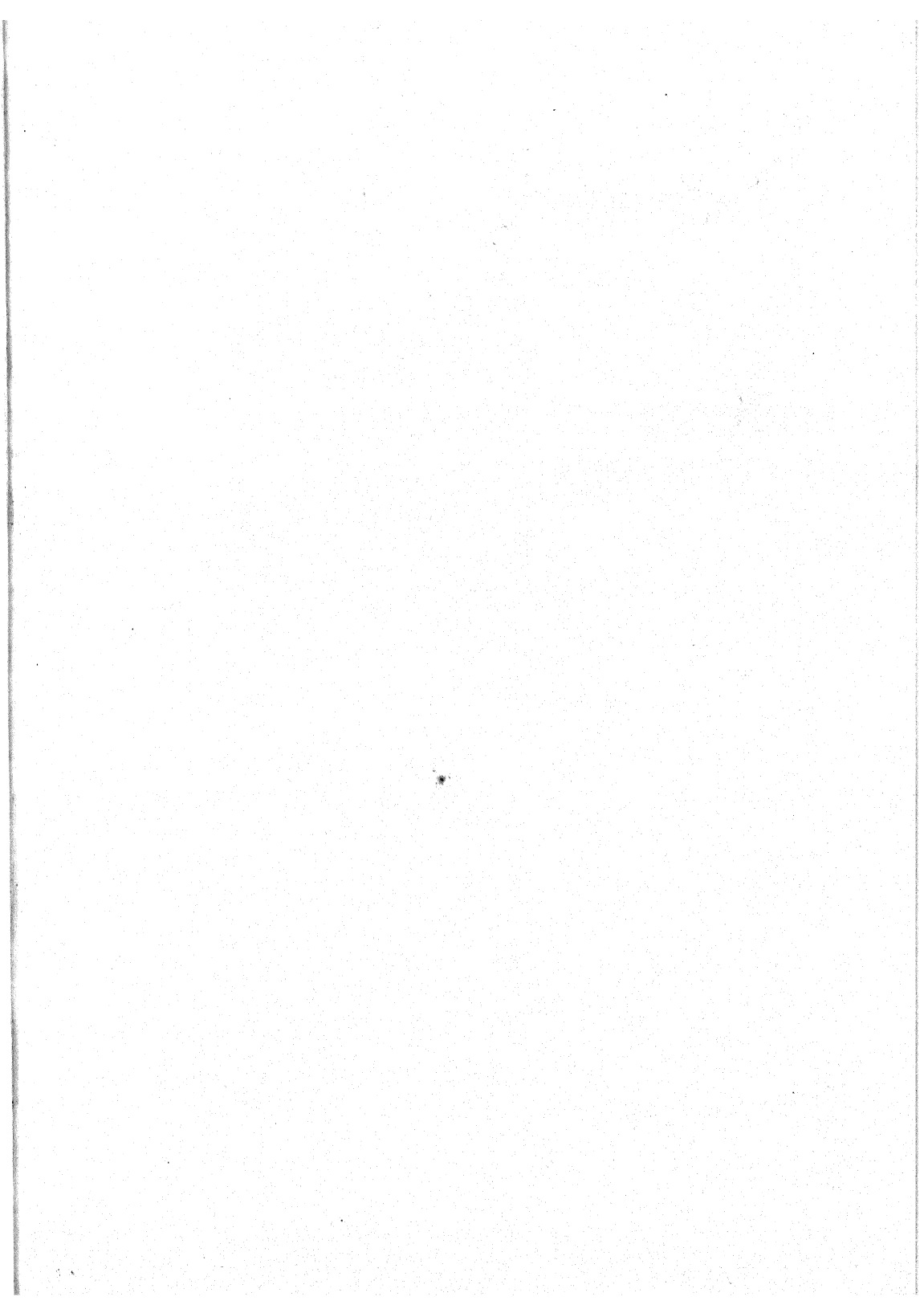
JUNE 25–26, INCLUSIVE

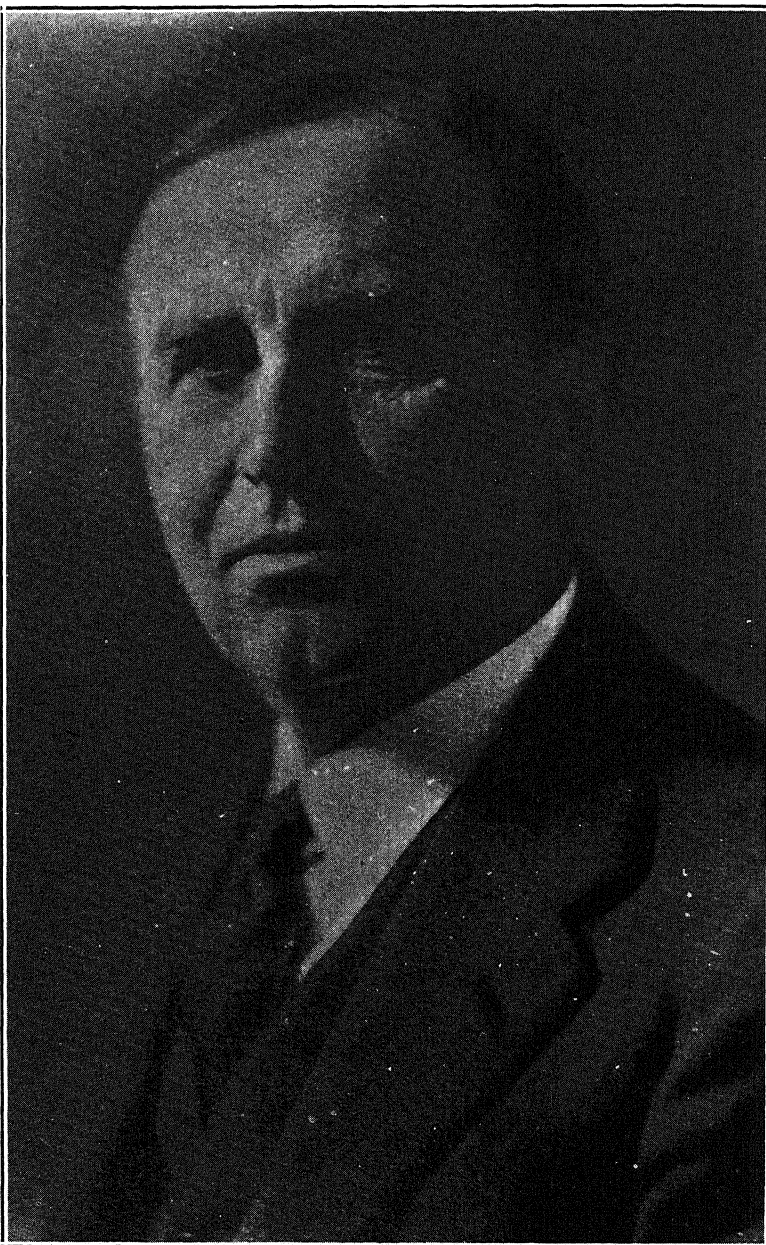
First Day

- 9:00 A.M.: Registration, Botany Hall, Room 202.
Tour through Laboratory and Greenhouses.
- 1:00 P.M.: Tour of Phytopathological Plots, Agronomy Farm,
Horticultural Orchards, and Federal Erosion
Nursery.
- 6:30 P.M.: Dinner, Memorial Union.
- 8:00 P.M.: Extension Workers' Round-table Discussion.
Coordination of Programs.
- 9:00 P.M.: Round-table Discussion—Research.
Relation of Phytopathology to Erosion Control.

Second Day

- 8:00–10:30 A.M.: Research—Round-table Discussion.
Is There a Place for Regional Research Programs in
Plant Pathology?
- 12:30 P.M.: Dinner, Leader Cafe, Kanawha.
- 1:30 P.M.: Excursion to Northern Iowa Experimental Farm,
Kanawha, Iowa—Cereals, Beets, Potatoes, etc.
Excursion to Southeastern Experimental Farm,
Conesville, Iowa—Melons, Sweet Potatoes, Other
Truck Crops.
Excursion to Western Iowa Fruit Section, Glenwood,
Iowa—Apple Spraying, Apple Root Rot, Nursery
Diseases.





JAMES M. VAN HOOK

PHYTOPATHOLOGY

VOLUME 26

JULY, 1936

NUMBER 7

JAMES M. VAN HOOK (1870-1935)

R. C. BUSTEED

James M. Van Hook, Professor of Botany in Indiana University, died at Pentwater, Michigan, June 20, 1935, after an illness of two years. His remains rest in the cemetery at Pentwater, Michigan. Survivors include the widow; a son; a daughter; a brother; and a sister.

Professor Van Hook was born in Washington County, Indiana, on December 19, 1870. For this early education Professor Van Hook attended the public schools with graduation from the Borden Institute of Borden, Indiana, in 1894. He attended Indiana University, where he received the A.B. degree in 1899, and the A.M. degree in 1900. He then entered Cornell University as a graduate student and assistant in botany, for a period of one year, and from 1902-04 he was assistant in plant pathology in the extension department of Cornell University. During his graduate work at Cornell he studied under Professor G. F. Atkinson. From 1904 until 1907 he was assistant botanist, Ohio Agricultural Experiment Station, with principal work in plant pathology, and was author of a number of station bulletins dealing with plant diseases. During his graduate work at Cornell, and later, at the Ohio Agricultural Experiment Station, much of his research was devoted to the disease of ginseng. The ginseng plant was, at that time, being grown extensively under cultivation, and the growers were confronted with a number of ginseng diseases, which were not well known.

That this young investigator, primarily a mycologist, could turn all the resources of his training, his knowledge, and his natural ingenuity to the practical problems of the control of plant diseases, was well demonstrated in his work on the diseases of ginseng. His findings were an important contribution to our knowledge of the diseases of ginseng and their control, and still stand as significant in its field.

In 1907 Professor Van Hook returned to Indiana University as assistant professor of botany. He was promoted through the various ranks, receiving the title of professor in 1925. At the time of his death he had thus completed almost three decades of service in that university.

During this period of service there were many students to teach, and a large part of this load fell to him. His teaching was characterized by an enthusiasm for his work, and an ability to impart this enthusiasm to the

interested student. He was acquainted with related fields as well as with his specific subject; and was able to present the facts and relationships clearly and concisely. He unselfishly shared his time and energy in and out of the classroom and laboratory, in personal sympathy and instruction. This seemed to him more important than fine-drawn definitions, and excessive theory in teaching. I am not aware that Professor Van Hook ever took a formal course in education, but I am certain he possessed the qualities of a highly successful teacher. His broad experiences and thorough training in mycology and plant pathology were brought to play in his teaching. That his ability was recognized and appreciated, was evidenced in the number of graduate students working under him for advanced degrees.

During his twenty-eight years of teaching at Indiana University his research was for the most part in mycology, with an occasional problem of diseases caused by fungi. His special field of study was with the Fungi Imperfecti. Unfortunately much of his work on this group remains unpublished. Prof. Van Hook, for the past 25 years, had been making a survey of the fungus flora of the State of Indiana, and, excepting the Uredinales, he contributed more to the knowledge of the fungi of Indiana than any other worker. The results of this survey have been published in part in the Proceedings of the Indiana Academy of Science.

In his work Professor Van Hook was opposed to the making of new species but believed where possible, a greater service could be rendered botanical science by a careful study of material collected in quantity and in various stages of development, and by appending corrections, and additions to already given meager descriptions. His extreme conservatism in naming new species is evidenced in the amount of his unpublished work on new species of imperfect fungi. His work on parasitic fungi was greatly enhanced by his familiarity with the taxonomy of the native host plants; he also was a specialist on ornamental plants.

By natural inclination, Professor Van Hook was of a quiet and retiring disposition. I believe few realize the extent and variety and thoroughness of his attainments. As a foundation he was well schooled in the classical languages and literatures and in music. He was a skilled craftsman. He brought these accomplishments to his service as a scientist. And through all he was a gentleman and a man of character.

His friendships were deep and lasting. Those who knew him recognized and admired his integrity, sincerity, kindness, and loyalty, and were impressed with his knowledge, skill, and ability in the field of botanical science, which he loved.

Professor Van Hook's interest in everything life presents made him a valuable member of his home and university community; made him an

enthusiastic cooperator in all efforts to make life more beautiful in every way and more enduringly satisfying.

Professor Van Hook was a fellow of the American Association for the Advancement of Science and of the Indiana Academy of Science; charter member of the American Phytopathological Society and of the Mycological Society of America; member of the Crop Protection Institute, the Botanical Society of America, Phi Beta Kappa and Sigma Xi.

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THE RELATION OF APHIDS TO THE TRANSMISSION OF BEAN MOSAIC¹

W. J. ZAUMEYER² AND C. W. KEARNS³

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INTRODUCTION

In practically all of the bean-growing regions of the United States, bean mosaic is a major factor in yield reduction, especially in some of the principal varieties. Although many species of insects have been mentioned as vectors of bean mosaic, no systematic search has been made of those feeding on beans in relation to the secondary spread of the virus. Bean mosaic is seed-borne, and it is through diseased seed that centers of infection are established. Investigations (6, 8) have shown that primarily infected plants seldom produce more than 50 per cent infected seeds, and secondarily infected plants even less. Seed transmission, although of extreme importance, fails to explain the rapid and widespread dissemination of mosaic in some regions during certain seasons. Fields planted with reasonably healthy seed stock often contain very high percentages of the disease at the end of the season.

This paper presents data on the prevalence of aphids in bean fields in relation to the spread of mosaic, together with data on the various species of aphids found on beans. Virus-transmission studies with 12 species of aphids, as well as with 10 other species of insects commonly collected in bean fields, also are described.

REVIEW OF LITERATURE

Nelson (5) was the first to report the transfer of bean mosaic by an insect. He succeeded in transferring the virus by *Macrosiphum solanifolii* Ashm., but was unable to transmit it with the bean leaf hopper, *Empoasca fabae* (Harris). Elmer (2) believed that the mealy bug, *Pseudococcus maritimus* Ehrh., was a vector and Fajardo (3) reported positive transmission of the mosaic virus by means of *Aphis rumicis* L., *Myzus persicae* Sulz., *Macrosiphum solanifolii*, and an undetermined species of mealy bug. He

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² Associate Pathologist, Bureau of Plant Industry.

³ Assistant in Entomology, University of Illinois, Urbana, Illinois, and formerly Agent, Bureau of Plant Industry, U. S. Department of Agriculture.

failed to transmit the virus by means of leaf hoppers, 12-spotted and striped cucumber beetles, red spiders, thrips, the capsid bug *Lygus pratensis* L., and a white fly, *Aleyrodes* sp. Pierce and Hungerford (8) transmitted the bean virus with an undetermined species of black aphid but not with a species of green leaf hopper, green aphid from alfalfa, and the tarnished plant bug. Merkel (4), who studied the mosaic diseases in the Papilionaceae, reports that *Aphis rumicis* and *Illinoia pisi* transmitted the viruses of *Phaseolus vulgaris* L., *Pisum sativum* L., *Lathyrus odoratus* L., *Lupinus luteus* L., *Melilotus altissima* Thu., *Trifolium hybridum* L., *T. repens* L., *Anthyllis vulneraria* L., and *Vicia faba* L. The results led the author to the conclusion that the same virus was responsible for all of the apparently different mosaics. Smith and Barker (9), in Haiti, report the transmission of bean yellows, resembling mosaic, by *Empoasca* sp. Zaumeyer (10) reported bean-mosaic transmission with *Macrosiphum solanifolii*, *Aphis rumicis*, *Myzus persicae*, *Illinoia pisi* Kalt., *Aphis gossypii* Glov., *Brevicoryne brassicae* L., *Hyalopteris atriplicis* L., and *Macrosiphum ambrosiae* Thos. Pierce (7) secured transmission with bean mosaic, as well as with a new virus known as yellow bean mosaic with both the pea aphid, *Illinoia pisi*, and the potato aphid, *Macrosiphum solanifolii*.

METHODS

The studies on the spread of mosaic in the field and also on the aphid populations found on beans were carried on both at Greeley, Colorado, and at Rosslyn, Virginia. In Colorado, 25 fields of the Stringless Green Refugee variety were chosen in which 2 plots, each containing 100 plants, were staked off in order to afford a means of checking weekly the presence of aphids and the development and spread of mosaic. Likewise, 100 plants were chosen at random every week in each field and the mosaic spread determined. In Virginia the data were collected in the experimental plots of the United States Department of Agriculture.

The transmission studies were carried out under cages in a greenhouse with temperature maintained at about 75–85° F. The aphids were collected in the field, where they were found colonizing on some particular host, or reared in the greenhouse. The following aphids were reared from single colonies under cages: *Aphis gossypii*, *Illinoia solanifolii*, *I. pisi*, and *Myzus persicae*. All of the other species used in the transmission experiments were collected from various host plants, as shown in table 3. These collections usually were made from individual plants within a species where the aphids were found colonizing in large numbers. In cases where it was believed a mixture of species occurred, the aphids were not used. Where the same species of aphid was found colonizing on several different host plants, as for example *A. rumicis*, which was collected from 6 different hosts, the aphids

were always identified from each host plant. All individual aphids were not identified, however a random sample of each collection was used for identification purposes. In no case was a mixture of species reported.

Aphids were then transferred to mosaic-infected Stringless Green Refugee seedlings, either by means of a camel-hair brush, or by removing portions of the plant upon which the aphids were abundant, and then later placing them on the leaves of the infected seedlings. The aphids migrated to the leaves of the bean plants and were allowed to feed for about 24 hours on the diseased ones. They were then retransferred to healthy seedlings of the Stringless Green Refugee variety by either of the two methods mentioned above. The pubescence of the dorsal side of bean leaves makes it difficult to transfer aphids by means of a camel-hair brush. However, by removing portions of the leaf upon which the aphids were feeding and placing them upon the healthy plant, little difficulty was encountered in the natural migration of the aphids. Since bean mosaic is not readily transferred except by rubbing healthy leaves with the expressed virus juice, there was no danger of transferring the virus if the leaves were carefully handled. The transfers were made under cages and the plants were allowed to remain there until mosaic symptoms appeared. Healthy check plants were placed adjacent to the cages in the open greenhouse.

Thrips, feeding on mosaic-infected bean plants, were collected in the field and transferred to healthy plants in cages by the same method described for aphids.

Leaf hoppers and other insects with sucking and chewing mouthparts were collected in the field by means of an insect sweeping net and placed in insect transmission vials. These vials containing 5 to 9 insects each were clipped on the foliage of diseased plants for a period of 1 to 8 days, and then the insects were transferred to healthy bean seedlings. Suitable check plants always were placed outside the cage.

PREVALENCE OF APHIDS IN BEAN FIELDS AND THE SPREAD OF MOSAIC

During the early part of the investigations, it was noted that very few aphids were caught by insect sweeps made in bean fields. Only after a careful examination was made of the underside of the leaves were aphids found to frequent beans in any numbers.

A careful examination of plants in the field indicates that beans apparently are not a favored host of aphids. Although many different species may be found on them, especially during the early part of the growing season, the writers have never found them in large numbers. The greatest infestation usually occurs about ten days after the beans are above ground. In Colorado they sometimes have averaged as many as 5 to the plant, but usually not more than 1 or 2. In the experimental plots at Rosslyn, Vir-

ginia, as many as 37 aphids were found on a single plant. As the season progresses, the populations decrease and, when the beans are mature, it is often difficult to find any.

It is believed that the aphids are transitory on beans and that they disappear when more favored hosts are found. Beans may serve only as a place for the aphids to obtain nourishment in their migrations. The writers have made numerous tests to determine whether aphids will colonize on beans and have found that if they are allowed to remain on beans from 48 to 72 hours they usually die. It is probable that the aphids do not long remain on a single plant, but in their search for a preferred host may visit many plants in a day. In doing this they may insert their mouthparts into the leaf and, finding it unfavorable, migrate to other plants.

Although no tests have been made to determine how long aphids must feed on a mosaic infected plant to become infective, it is believed that they acquire the virus on their first feeding and are able to infect healthy plants immediately. If the incubation period were longer it is believed little transmission of the mosaic would take place since, as pointed out, aphids cannot live on beans for any length of time.

SPECIES OF APHIDS FOUND ON BEANS IN THE FIELD

Although aphids are not found in large numbers nor throughout the entire growing season, the following species have been collected from beans: *Hyalopterus atriplicis* L., *Aphis gossypii* Glov., *Aphis medicaginis* Koch, *Aphis rumicis* L., *Illinoia pisi* Kalt., *I. solanifolii* Ashm., *Myzus persicae* Sulz., *Brevicoryne brassicae* L., *Myzocallis ononidis* Kalt., *Monellia* sp., *Calaphis* sp., *Aspidaphis* sp., *Macrosiphum* sp., and *Tritogenaphis* sp. A careful search would probably reveal many other species.

In 1931, at Rosslyn, Virginia, a collection was made of the various species of aphids on beans. The potato aphids, *Illinoia solanifolii*, accounted for 60 per cent, the bean aphid, *Aphis rumicis*, for about 20 per cent, the pea aphid *I. pisi*, for 10 per cent, the peach aphid, *Myzus persicae*, for 5 per cent, and the remaining species for 5 per cent of the aphids collected. It is quite probable that the relative percentages of the different species depend to a large extent on the crop growing in close proximity to bean fields. In Colorado, *Hyalopterus atriplicis*, *A. medicaginis*, and *A. rumicis* were found most commonly on beans. *Hyalopterus atriplicis* feeds to a large extent on *Chenopodium album* L., a common weed found in the cultivated fields of northern Colorado. *A. medicaginis* is a general feeder, but is found feeding largely on legumes such as alfalfa, *Medicago sativum* L., sweetclover, *Melilotus alba* Desr., and yellow sweetclover, *Melilotus officinalis* L. These hosts grow both under cultivation and wild along fence rows and irrigation ditches. *A. rumicis* is found to a great extent feeding on beet, *Beta vulgaris* L., an important crop plant in Colorado.

TRANSMISSION OF BEAN MOSAIC BY APHIDS

Although a few species of aphids were shown by other investigators (3, 4, 5, 6, 8) to be able to transmit the bean-mosaic virus, they never have been found numerous enough in bean fields to be suspected as primary vectors of the disease. Since many species were found feeding on beans in the field, tests were made to determine whether a number of different species would transfer the virus from diseased to healthy plants.

Owing to insufficient numbers, it was not possible in every case to use in these studies all of the species found on beans. Of the 14 species found on beans, 6 were not collected in large enough numbers for testing. They are as follows: *Myzocallis ononidis*, *Monellia* sp., *Calaphis* sp., *Aspidaphis* sp., *Macrosiphum* sp., and *Tritogenaphis* sp. Furthermore, 4 other species, not found on beans, also were used. Since aphids in general do not colonize on beans in any appreciable numbers, the 12 species used were collected in sufficient numbers from various hosts, where they were readily found.

The aphids used in the transmission studies were collected from 17 hosts as follows: *Aphis gossypii* from *Cucumis* sp., *Aphis medicaginis* from *Amaranthus retroflexus* L. and *Phaseolus lunatus* L., *Aphis rumicis* from *Trifolium repens* L., *Papaver omniferum* L., *Dahlia* sp., *Nasturtium* sp., *Rumex crispus* L., and *Cosmos* sp., *Aphis spiraeicola* from *Spiraea vanhouttei* Zabel, *Brevicoryne brassicae* from *Nasturtium* sp., *Hyalopecterus atriplicis* from *Chenopodium album*, *Rhopalosiphum pseudobrassicae* Davis from *Brassica rapa* L., *Macrosiphum ambrosiae* Thos. from *Aster* sp., *Illinoia solanifolii* from *Solanum tuberosum* L., *Illinoia pisi* from *Pisum sativum* L., *Myzus persicae* from *Amaranthus retroflexus* L., and *Neothomasia populicola* Thos. from *Populus deltoides* Marsh. Table 1 shows the results of bean mosaic transmission with the above named species, under greenhouse conditions.

It is apparent from table 1 that all of the aphid species studied can transmit bean-mosaic virus from diseased to healthy plants, with the exception of *Neothomasia populicola*, collected from cottonwood. As previously pointed out, not all of the species used were collected from beans; but it is likely that many of them would be found on the host if extended searches were made. Since ability to transmit the virus is not necessarily confined to one species of aphid, it is likely that numerous other species not tested also would be able to spread the virus.

PREVALENCE OF APHIDS IN RELATION TO THE SPREAD OF MOSAIC

Since bean mosaic is seed-borne, the infective principle is available to the aphids as soon as the plants appear above ground and it is at this time that aphids are most numerous on beans. A short time after aphids feed

TABLE 1.—*Results of cage experiments on aphid transmission of bean mosaic^a*

Species of Aphid	Host	Plants inoculated	Plants diseased	Infection	Check	Checks diseased
<i>Aphis gossypii</i>	Cucumis sp. ^b	62	38	<i>Per cent</i>	12	0
<i>Aphis medicaginis</i>	{ <i>Amaranthus retroflexus</i> ^b <i>Phaseolus lunatus</i> ^b	27	12	61	12	0
<i>Aphis rumicis</i>	{ <i>Trifolium repens</i> ^b <i>Papaver somniferum</i> ^b <i>Dahlia</i> sp. ^b <i>Nasturtium</i> sp. ^c <i>Cosmos</i> sp. ^b <i>Rumex crispus</i> ^c	110	79	44	12	0
<i>Aphis spiraeola</i>	<i>Spirea vanhouttei</i> ^b	30	26	72	12	0
<i>Brevicoryne brassicae</i>	<i>Nasturtium</i> sp. ^b	14	8	87	12	0
<i>Hyalopterus atriplicis</i>	<i>Chenopodium album</i> ^b	15	14	57	12	0
<i>Rhopalosiphum pseudobrassicæ</i>	<i>Brassica rapa</i> ^b	10	10	93	12	0
<i>Macrosiphum ambrosiae</i>	<i>Aster</i> sp. ^b	36	28	100	12	0
<i>Illinoia solanifolii</i>	<i>Solanum tuberosum</i> ^c	83	48	79	12	0
<i>Illinoia pisi</i>	<i>Pisum sativum</i> ^c	50	26	59	12	0
<i>Myzus persicae</i>	<i>Amaranthus retroflexus</i> ^c	28	23	52	12	0
<i>Neothomasia populicola</i>	<i>Populus deltoides</i> ^b	19	0	82	12	0
Average per cent infection.....		484	312	0	144	0
Total		484	312	64	144	0

^a The Stringless Green Refugee variety was used in all experiments.^b Aphids from these hosts identified by P. W. Mason, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.^c Aphids from these hosts identified by W. J. Zaunmeyer.

on the plants they become a source of infection. The secondary spread of mosaic in fields containing high percentages of seed-borne mosaic is usually very rapid, while in fields containing a small amount, the disease may not increase. There apparently is a direct correlation between the size of aphid populations and the amount of mosaic spread. However, a small population in fields containing a high percentage of seed-borne infected mosaic plants may spread the disease more rapidly than a large population of aphids in a field containing a small percentage of seed-borne mosaic.

Table 2 shows data collected at Greeley, Colorado, and Rosslyn, Virginia, on the spread of bean mosaic in relation to the prevalence of aphids throughout most of the growing season. At Greeley the data on the percentage of mosaic and the number of aphids per plant were based on the

TABLE 2.—*Data showing mosaic spread and the prevalence of aphids on beans^a*

Location	Date	Number of plants	Percentage of mosaic	Per cent weekly increase	Ratio of aphids to plants
Greeley, Colo., 1930 ^b	6/20	300	7.0	1:1
	6/27-30	300	15.7	8.7	1:1
	7/3-7	300	19.4	3.7	1:2
	7/12-14	300	30.3	10.9	1:7
	7/19-22	300	38.9	8.6	1:14
	7/26-28	300	41.3	2.4	Few
	8/1-4	300	41.8	0.5	None
Total mosaic increase				35.6	
Rosslyn, Va., 1931	6/15	320	5:1
	6/18	320	2:3
	6/22	320	13.0	1:1
	6/29	320	50.0	37.0	1:1
	7/6	320	72.4	22.4	1:5
	7/13	320	97.0	24.6	1:2
	7/20	320	99.0	2.0	1:40
Total mosaic increase				86.0	

^a Stringless Green Refugee variety.

^b Data based on average of 300 plants in each of 25 fields.

average of 300 plants in each of 25 fields. At Rosslyn, the data were obtained from 320 plants in one plot.

It is evident from table 2 that, although the aphid population on beans was relatively small at Greeley in 1930, and at Rosslyn in 1931, the spread of mosaic was considerable. The difference between Greeley and Rosslyn in regard to the percentage increase is unquestionably due to the difference in the prevalence of aphids, although it is probable that there was more seed-borne infection in the beans grown in Virginia than in those grown in Colorado.

As the season progressed the aphid populations decreased. In Colorado, on June 20, the aphids averaged 1 per plant and from August 1-4 no aphids were found. A similar decrease occurred at Rosslyn, Virginia. On June 15, the aphids averaged 5 per plant and on July 20, the last date observations were made, there was an average of 1 to 40 plants.

Under field conditions the mosaic symptoms are noticeable about 10 to 12 days after inoculation, although the plant may act as a source of infection long before this. From table 2 it can be seen that, in Colorado, mosaic increased 8.7 per cent from June 20 to June 27-30, whereas during the following week the increase was only 3.7 per cent.

The aphids feeding during the week preceding June 20 are probably responsible for the spread of mosaic reported on June 27-30. Those feeding on beans from June 20 to June 27-30 account for the mosaic for July 3-7. Since the average number of aphids found on June 20 and for the following week were approximately the same, it would be assumed that the mosaic increase on July 3-7 should be equally as great as, if not greater than, for the week of June 27-30.

This difference in the actual mosaic spread and the expected spread of the disease may be accounted for in one of two ways. On June 20, when the first mosaic reading was made in Colorado, the beans were in the primary-leaf stage, at which time mosaic symptoms are difficult to diagnose. It is probable that, because of this, some infected plants were overlooked and actually a higher percentage of mosaic was present on June 20 than the amount recorded. If this were true, the percentage of mosaic increase for the week of June 27-30 would have been less than 8.7 per cent. Likewise, the aphid population may have been greater during the week preceding June 20 than those recorded for this date. This could have accounted for a considerable spread of mosaic appearing about June 27-30 and a decrease the following week because of a reduced aphid population.

The greatest mosaic increase in Colorado was noted from July 12-14 about 10 days after a relatively large aphid population, together with a considerable amount of mosaic in the field. The weekly spread of mosaic and aphid population continued to decrease from this date until the experiment

TABLE 3.—Data showing spread of mosaic in relation to prevalence of aphids and seed-borne infections^a

Date	Field No. 1		Field No. 2		Field No. 3		Field No. 4	
	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants
6/21	0	1:6	0.3	1:3	0.3	1:2	0	2:3
6/30	0.6	1:6	0.3	2:1	0.3	1:1	2.0	1:3
7/7	1.3	1:10	0.5	1:6	1.3	1:3	2.3	1:3
7/14	2.0	None	4.3	1:6	6.0	1:4	9.0	1:6
7/18	4.7	"	11.3	1:10	12.0	1:5	20.0	1:6
7/25	7.0	"	12.0	None	17.3	1:8	30.0	1:8
8/1	8.0	"	13.0	"	19.7	None	31.3	None
Total mosaic increase	8.0		12.7		19.4		29.3	

^a The data recorded in each field are based on 300 plants of the Stringless Green Refugee variety.

TABLE 3—(Continued)

Date	Field No. 5		Field No. 6		Field No. 7		Field No. 8	
	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants
6/21	1.5	1:3	14.5	1:2	13.0	1:1	4.0	2:1
6/30	12.0	1:4	35.0	1:8	26.3	1:2	20.0	1:1
7/7	12.3	1:8	36.7	1:4	36.0	1:3	42.0	2:3
7/14	17.3	1:10	47.0	1:8	46.0	1:6	67.7	1:3
7/18	25.0	None	57.3	1:10	51.3	1:6	72.7	1:6
7/25	28.7	"	58.7	None	52.3	None	73.0	1:10
8/1	29.0	"	60.3	"	59.7	"	73.0	None
Total mosaic increase	27.5		46.8		47.3		69.0	

was terminated. Because of the increased number of infected plants, fewer aphids are required to transmit a reasonably high percentage of mosaic later in the season, while, at the same time, the decrease in number of aphids apparently accounted for the decrease in secondary spread.

The data obtained at Rosslyn, Virginia, while showing a greater secondary spread of mosaic, are similar to those taken in Colorado. Mosaic symptoms were first observed on June 22, and increased a week later by 37 per cent. This was due possibly to the large aphid population about 2 weeks previously, which averaged 5 to the plant. Since there was considerable initial infection, the spread was rapid. The number of aphids decreased, later accompanied by a decrease in the secondary spread.

This decrease in secondary spread of mosaic late in the season, especially in fields containing a high percentage of infection, may not have been entirely due to a decrease in number of aphids. Since aphids presumably feed at random, the chances of their feeding on healthy plants after becoming infective would necessarily decrease as the proportion of mosaic plants increased. It is apparently for this reason as well as for a decrease in number of aphids that the secondary spread of mosaic does not increase so rapidly late in the season as it does earlier.

The secondary spread of mosaic is dependent not only on the number of aphids present but also on the amount of seed-borne infection present early in the season. In this connection, 8 bean fields were chosen to study the relationship of mosaic spread to aphid prevalence and seed-borne mosaic infection. The amount of mosaic in these fields at the beginning of the study ranged from 0 to 14.5 per cent and the aphid populations from relatively small to reasonably larger. The results of the observations are recorded in table 3.

In comparing the mosaic increase in field No. 1 with that of field No. 4, it can be seen that the spread in the latter is almost 4 times as great as in the former. In both fields no mosaic was evident on June 21. The aphid populations in field No. 1 were less throughout the season than in field No. 4, which accounted for this difference in the spread of mosaic. In fields No. 2 and No. 3, where the amount of mosaic infection was only 0.3 per cent on June 21, the mosaic had increased to 12.7 per cent in field No. 2 and to 19.4 per cent in field No. 3. Although the aphids were more numerous in the former field on June 30 than in the latter, more were present in field No. 3 than in field No. 2 throughout the entire season.

A comparison of field No. 5 with No. 8 shows that although the initial amount of mosaic infection was slightly greater in the latter than in the former, the amount of secondary spread was much greater in field No. 8 than in No. 5. This can be explained by the larger aphid population in field No. 8 than in field No. 5. In field No. 5 the largest population of aphids aver-

aged 1 for every 3 plants on June 20. After July 22 no aphids were present. On the other hand, in field No. 8 the aphids averaged 2 to the plant in the early part of the season and some were present as late as July 25.

The plants in fields No. 6 and No. 7 had approximately the same amount of initial infection on June 20 and about the same amount of secondary spread at the time when the data were completed. The aphid populations in both fields varied slightly, but, in general, they were approximately the same. Field No. 6, which had a greater amount of primary infection and fewer aphids than field No. 7, showed less secondary mosaic spread.

The evidence brought out in the eight fields, table 3, shows that with a small amount of seed-borne mosaic, present early in the season, a reasonably small population of aphids spread mosaic less rapidly than a larger population. On the other hand, a small population of aphids present in a field with a considerable amount of primary mosaic will spread very rapidly. A large aphid population in a field with a small amount of mosaic in the early part of the season produces considerable spread of the disease. This is shown in field No. 8, where, on June 20, there was 4 per cent mosaic with a population of two aphids per plant. Nine days following this the mosaic had spread to 20 per cent and by August 1, there was 73 per cent mosaic.

The writers and previous investigators have observed that mosaic is usually more severe along the borders of the field than elsewhere. In numerous instances as much as 15-20 per cent infection has been noted in the outermost rows, where only a trace was observed in the central portion of the field. This point was clearly demonstrated in two particular instances where detailed records were made on the weekly spread of mosaic in the Stringless Green Refugee variety. Two plots of 100 plants each were staked off in two fields and mosaic counts were made each week. In field No. 1 the first plot was located adjacent to an alfalfa field, while the second was near the center of the field. In field No. 2 the first plot was planted close to a field of rye seeded to alfalfa, while the second was also near the middle of the field. In both fields, plot No. 1 showed considerably more secondary spread than the plots located in the center of the bean field. In plots No. 1 and No. 2 of field No. 1 there were 3 per cent and 2 per cent of mosaic, respectively, on June 21 when the first mosaic reading was made. On August 1, when the experiment was terminated, the beans in plot No. 1 had 41 per cent mosaic, while in plot No. 2 the disease had increased to 18 per cent. The mosaic in plot No. 1 of field No. 2 had increased from 0.5 per cent to 24 per cent from June 21 to August 1, while the mosaic in plot No. 2, located in the center of the field, had increased only 7 per cent during the same time. The increases of mosaic spread of 23 per cent between the two plots in field No. 1 and of 17 per cent in field No. 2 are undoubtedly accounted for by the presence of larger numbers of aphids migrating from alfalfa and rye fields to the beans in the border rows.

Although no detailed records have been made to correlate the insect populations found in border crops and weeds with those found in the outermost rows and in the center of bean fields, observations indicate that early in the growing season more insects occur in the outermost rows than elsewhere in the field. Later in the season the insects seem to be numerous in all portions of the field. Regarding the prevalence of aphids, it appears that the crop growing adjacent to a bean field is in general correlated with the number and species of aphids found in bean fields. It is likely that larger populations of aphids would be found on beans, especially if they were planted adjacent to a leguminous crop such as alfalfa or sweetclover. Since these crops appear early in the spring they afford an ideal feeding and breeding place for aphids. They may migrate later to other near-by crops. Since it has been shown (11) that the virus diseases of a number of legumes are transmissible to beans producing symptoms that are often difficult to distinguish from the common bean mosaic, the increased severity of mosaic along the field borders may be accounted for in two ways; either by the transmission of the virus from these legumes to beans by aphids, or by the spreading of the common bean mosaic from one plant to another.

Prevalence of Other Insects on Beans⁴

A systematic search was made for other insects on beans in Colorado, to determine if some of them might also be responsible for the secondary transmission of the virus. Some years the flea beetles *Epitrix cucumeris* Harris, and *E. fuscula* Crotch, the Mexican bean beetle, *Epilachna corrupta* Muls., and the grasshopper *Melanoplus* sp. are very numerous on beans, often causing severe damage in certain sections.

Many other species of insects were observed on beans, a few of the more common ones being: The clover leaf hopper, *Aceratagallia sanguinolenta* (Prov.), the bean leaf hopper, *Empoasca fabae* (Harris), *E. filamenta* DeL., the tarnished plant bug, *Lygus pratensis* L., *L. elysius* Van D., the false chinch bug, *Nysius ericae* Schilling, the striped cucumber beetle, *Diabrotica vittata* Fab., *Thrips* sp., and many others.

Transmission studies were conducted with all of these insects, except the Mexican bean beetle and the grasshopper, and all yielded negative results. This confirms the reports of other workers (3, 5, 8) who were unable to transmit the virus with a number of the species recorded here. The number of plants on which insects fed ranged from 5 to 66, and the numbers of insects allowed to feed on infected plants and later transferred to healthy ones were from 5 to 13 per plant. Although further tests may show that some of them, as well as others not tested, may be vectors of the bean-mosaic virus, it appears that they play little part in the wide-spread dissemination of the dis-

⁴ Insects mentioned under this heading identified by C. W. Kearns.

ease under field conditions. This is especially true of the two species of flea beetle and the clover leaf hopper, both of which are usually very numerous on beans in the early part of the season, and of the Mexican bean beetle and the grasshopper, which appear later. If any of these insects were vectors of the virus, all of the susceptible bean varieties would possibly be 100 per cent infected by the end of the season.

DISCUSSION

The evidence presented shows that although aphids are not so numerous on beans as many other species of insects, they are responsible for the dissemination of mosaic under field conditions. Because of the difficulty in collecting aphids by means of the usual field methods and in finding them except by careful examination of the individual plants, they have not been heretofore suspected as vectors in the transmission of bean mosaic under field conditions.

Because of the fact that aphids do not readily colonize on beans, many workers have concluded that they were not responsible for the field dissemination of the disease. Although Nelson (5, 6) is of the opinion that aphids are able to transmit bean mosaic, he believes that none of those tested by him are concerned with the wide-spread dissemination of the disease in Michigan. Likewise, Pierce and Hungerford (8) were not able to show any connection between the spread of mosaic and the prevalence of aphids under Idaho conditions, although they showed them to be vectors.

From the evidence presented on the prevalence of aphids in relation to the spread of mosaic (Table 3), it hardly seems possible that so few aphids could account for the wide-spread dissemination of the disease as the results indicate. Although the numbers of aphids recorded in the weekly examinations both in Colorado and Virginia do not indicate an abundance of these insects for any particular date, it is probable that their numbers may often have been larger between these periods. It is important to remember that there apparently was a continuous drift of winged aphids into the field and that those present on the plants when the counts were made had not been there over 72 hours or, as indicated in the rearing studies, they would have been dead. In view of this fact, it seems reasonable to conclude that the weekly aphid population counts represented the number of aphids present on a particular date rather than the total weekly aphid population. In other words, the total population for any week would be considerably larger than indicated by the weekly counts. It is likely that an average of 1 aphid per plant recorded for any single date may have represented the presence of 7 or more per plant for the entire week.

Most of the aphids found on beans were the winged migrant forms; and, since the bean is not a preferred host, the migration is likely to be continu-

ous. Furthermore, aphids in any appreciable numbers coming from other hosts and feeding on beans probably would not remain there but a short time, but would migrate to more favored hosts. Before doing so, however, they may become infective and feed on a number of plants in various parts of the field, transmitting the virus and thereby causing a rather general spread of the disease. Since Fajardo (3) has shown that a single aphid is able to transmit the virus, it is not unlikely that even a small population moving frequently from plant to plant could transmit the disease to many plants in the course of a short time. Furthermore, since the ability to transmit the bean mosaic virus is not necessarily confined to a single species of aphid, but the virus can be transmitted by many species, it appears that they are responsible for the field dissemination of the disease.

Both Elmer (2) and Fajardo (3) believe the mealy bug, *Pseudococcus maritimus*, is a vector of the virus. In northern climates these insects occur mainly in greenhouses, but they have been reported as occurring out of doors, especially on the roots of certain perennial crops. It is believed that they are not apt to be found feeding on beans in the field and would not, therefore, play a part in the field dissemination of the disease.

Smith and Barker's (9) studies indicate that the bean yellows, transmitted by *Empoasca* sp., is apparently unlike the common bean mosaic in this country. Previous to the report of Drake (1) *et al.*, who found over 50 different species of aphids able to transmit the yellow dwarf of onions, aphids in general were considered quite specific in the transmission of the various virus diseases. Since 11 different species of aphids have been shown to transmit bean mosaic, it is quite likely that others also will prove to be vectors. Furthermore, extended field studies on the insect fauna of beans may reveal many more species of aphids present on beans than those recorded in a single year's survey.

SUMMARY

The studies presented show that aphids are not found in large numbers feeding on beans in the field. The greatest infestation usually occurs about 10 days after the beans are above ground. As the season progresses, the populations decrease.

Fourteen species of aphids were found on beans in the field and it is likely that a careful search would reveal many others. Not all of the species found were tested, since, in some cases, they were too few. It is probable that the relative percentages of the different species found on beans depend to a large extent on the crop growing in close proximity to bean fields.

Positive transmission of the bean-mosaic virus was proved for 11 species of aphids collected from 17 different host plants. Only one of the aphid

species tested (*Neothomasia populicola*) collected from the cottonwood tree gave negative results.

The spread of the bean mosaic in the field is dependent on the prevalence of aphids, as well as on the amount of primary infection appearing early in the season.

Transmission studies with 10 species of insects other than aphids commonly found feeding on beans yielded negative results.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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FACTORS INFLUENCING INFECTION OF BARLEY BY LOOSE SMUT

R. W. L E U K E L ¹

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INTRODUCTION

In an earlier paper the writer (4) presented the results of experiments designed to determine the influence of certain environmental factors on the development of loose smut in barley. At that time it was taken for granted that there existed only one species of loose smut attacking barley. The wide differences in results obtained on the control of this loose smut by means of chemical seed treatments were attributed to differences in the manner of natural inoculation by the causal fungus on different varieties of barley. Tapke (6, 7) has since described another loose smut of barley and has named it *Ustilago nigra* Tapke.² Two of the chief characteristics distinguishing it from *U. nuda* are its ability to cause infection when spores are applied to the seed and its amenability to control by surface disinfectants.

The existence of two loose smuts of barley, one amenable to control only by the hot-water treatment and the other by ordinary seed disinfectants, explains many of the conflicting data presented in recent years on the loose smut of barley and its control. The work of developing varieties of barley resistant to this newly discovered loose smut demands a knowledge of what environmental conditions are most conducive to its development, so that supposedly resistant varieties may be tested under these conditions.

Since previous experiments dealing with the effects of environmental factors during the early life of the plants on the incidence of barley loose smut leave one in doubt as to which loose smut was involved, further work of this nature was done with *Ustilago nigra*.

REVIEW OF LITERATURE

Considerable work has been done by various investigators on the effect of environmental conditions on infection by other cereal smuts. This includes studies of the factors influencing the germination of both the smut spores and the cereal seeds. Attempts have been made to correlate slow seed germination and tardy host-seedling emergence with abundant smut infection. Much of this work has dealt with wheat and the organisms causing bunt. The smuts of oats and sorghum and the covered smut of barley also

¹ The writer gratefully acknowledges the advice and assistance of Dr. V. F. Tapke throughout the progress of these investigations.

² Some investigators are in doubt as to whether this smut should be accorded specific rank. In this paper, however, it will be referred to as *Ustilago nigra*.

have received considerable similar study. The salient literature on this subject has been reviewed by Reed and Faris (5). Loose smut of barley, on the other hand, has been largely omitted in studies of this kind. As previously stated, such studies as have been made on this smut are, in the light of Tapke's discovery, now of uncertain value because it is not definitely known which loose smut of barley figured in the experiments, or whether both may not have been present.

MATERIALS AND METHODS

Alpha barley was the principal variety used in these experiments, although Wisconsin Pedigree No. 5 was employed in a few cases. The first lot of Alpha used for work with *Ustilago nigra* unfortunately (unknown to the writer) carried some natural inoculum of both species of loose smut. This made the results obtained harder to interpret. The second lot of Alpha seed was raised in the greenhouse and was smut-free. The smut used for inoculation was obtained in the greenhouse, from mature plants that were allowed to cure and dry thoroughly before the smutty heads were removed. The latter were rubbed through a 60-mesh sieve and the powdered smut kept in a glass vial at 7° C.³ until used.

Two methods of inoculating the seed were used. The first was a modification of the "spore suspension-vacuum method" employed in Germany and described by Haarring (2). A spore suspension was made by thoroughly shaking up 1 gram of spores in 1000 cc. of 1 per cent dextrose solution. This amount of spore suspension was poured over 250 grams of seed and the container again thoroughly shaken. Then by means of a small motor-driven vacuum pump the barley, together with the spore suspension, was subjected to 35 inches of vacuum for 20 minutes while being shaken occasionally to facilitate escape of air. The vacuum was then released, the liquid drained off, and the seed allowed to dry overnight. It was then stored for about 2 days at 25° C. and about 90 per cent relative atmospheric humidity.

The other method employed consisted simply in dusting the seed with dry spores and then incubating it as above.

Soil Moisture

Exact percentages of soil saturation are difficult to secure and maintain and no serious attempt was made to adjust the soil to predetermined exact percentages of its water-holding capacity. The aim rather was to obtain a relatively dry soil, a relatively wet soil, and one about half saturated. The water-holding capacity of the soil was first determined and then its actual moisture content. Sufficient water then was added to a given weight of soil to bring it up approximately to a given percentage of saturation. In

³ The Centigrade temperature scale is used throughout this paper.

order to prevent puddling in the case of the very wet soil, the final increment of water was not added until after the seed had been planted. The soil cans were covered until after emergence to prevent evaporation.

In experiments carried out in the greenhouse bench, adjustments of the dry and medium-wet soils were secured by several tests. The wet-soil adjustment was obtained after planting by soaking the soil in the bench twice daily. The other sections were covered with heavy canvas until the seedlings emerged. Water was withheld from the dry and medium-wet sections until the plants were in the second-leaf stage. During this period the wet section was watered heavily daily.

Soil Temperature

A range of soil temperatures was secured by means of the soil-temperature tanks previously described (3). These tanks, although still the same in principle, had been improved by the addition of electric refrigeration and more efficient control instruments. In certain experiments controlled temperature chambers were employed. The low temperatures were automatically maintained by means of a compressor, refrigerating coils, and multiple expansion valves.

The air temperature in the greenhouse and consequently also the soil temperature in the benches ranged from 15 to 25° C., which, as will be shown later, is a range more or less favorable for infection by *Ustilago nigra*.

The plants grown in the soil cans suspended in the tanks were transferred to the greenhouse bench when they had reached the second-leaf stage.

The plants in the temperature chambers were grown in glass-covered flats. On emerging they were subjected to artificial illumination until large enough to transplant. Some time after the plants had been transferred to the bench, the regular daylight period was supplemented by electric lights, so that an 18-hour day resulted, thus hastening maturity.

Soil Composition

The possible influence of soil composition on infection was studied by growing artificially inoculated Alpha barley in 3 kinds of soil: (1) river sand, (2) Keyport silt loam from the Arlington Experiment Farm, and (3) a rich black "garden soil" to which leaf mold and powdered sheep manure had been added. The sand, loam, and "garden soil" had water-holding capacities of 21, 35, and 50 per cent, respectively, and the moisture content of each was adjusted to about 65 per cent of its respective water-holding capacity. The first experiment was carried out in deep flats and the second in 3 sections of greenhouse bench. The greenhouse temperature ranged from 10° to 20° C. during the first experiment and from 15° to 25° C. during the second.

SOIL REACTION

To obtain a range in soil reaction, dilute sulphuric acid or calcium carbonate was added to a good grade of top soil with an initial pH of 6.6. By preliminary tests it was determined how much acid or calcium carbonate to add to a given weight of this soil to produce the desired soil reaction. After the proper amount of calcium carbonate or acid had been added, the different lots of soil were shoveled over frequently during a period of several weeks and tested each time for reaction by means of the La Motte colorimetric test and also by means of the W. A. Taylor slide comparator. The process of adjusting these lots of soil to different reactions was a long, laborious one requiring several months.

The first experiment was carried out in deep flats and the other two in sections of greenhouse bench.

EXPERIMENTAL RESULTS

Soil Moisture and Temperature

The results from experiments involving differences only in soil moisture are shown in table 1. In the first experiment, in which the maximum percentage of saturation was only 75 per cent, there were no significant differences in the percentages of infection obtained in the 3 lots of soil. In the second experiment significantly less smut developed in the very wet soil than in the other 2 lots. The same held true in the third experiment in the case of Alpha barley, but in Wisconsin Pedigree No. 5 the differences were barely significant. In none of these experiments were there any significant differences between the percentages of smut obtained in the soils containing low and medium amounts of moisture, although in earlier experiments (4) a minimum of soil moisture had seemed most conducive to smut development.

Additional data on the effect of differences in soil-moisture content on infection were obtained in connection with experiments involving also soil-temperature differences and are presented in table 2.

The lowest percentages of infection, especially at 5° and 30° C., and, with two exceptions, also at the other temperatures, occurred in the very wet soil. The plants grown to emergence in relatively dry soil at 5° C., contained much more smut than did the plants in the wetter soils at the same temperature. This was not the case at the higher temperatures, and is suggestive of the "interaction of factors" mentioned by Reed and Faris (5).

Differences between 10 and 25° C. in soil temperatures during emergence had no great influence on the percentage of infection. The highest average percentages of infection were secured at 15 and 20° C., with moderate declines at 10 and 25°, a greater drop at 5° and the most pronounced decrease at 30°. In 4 cases excessive soil moisture, combined with high or low temperature,

TABLE 1.—Effect of soil moisture on infection by loose smut in Alpha and Wisconsin Pedigree No. 5 barleys grown from seed artificially inoculated with spores of *Ustilago nigra* and sown in soil adjusted to different percentages of its water-holding capacity

Experiment	Variety	Range of soil temperature	Soil saturation		Plants		
			After sowing	After emergence	Grown	Smutted	
No.		° C.	Per cent	Per cent	No.	No.	Per cent
1	Alpha	10 to 20	30	25	392	77	19.6
			50	45	381	74	19.4
			75	73	442	98	22.2
2	Alpha	15 to 25	33	20	271	258	95.2
			57	44	286	257	89.9
			85	a	270	174	64.4
	Alpha	15 to 25	25	19	237	207	87.3
			45	39	244	207	84.8
			85	a	235	128	54.5
3	Wisconsin Pedigree No. 5	15 to 25	25	19	284	203	71.5
			45	39	282	209	74.1
			85	a	266	174	65.4

^a Kept near saturation by watering heavily daily.

eliminated smut. Although in several cases rather high percentages of infection (59 and 73 per cent) occurred at 5°, due, possibly, to an unobserved irregularity in temperature, it is evident that 5° and 30° are much less conducive to infection than are the intermediate temperatures.

The data in series I (Table 2) are more or less affected by the presence of an unknown amount of smut due to *Ustilago nuda* with which the seed was, to some extent, naturally inoculated. In order to determine experimentally the approximate amount of infection by *U. nuda* so as to be able to interpret the results, and also to compare the effectiveness of the two methods of inoculation used, the following experiment was carried out. A quantity of the Alpha barley seed in question was divided into 4 lots: Lot 1 was neither inoculated nor treated; lot 2 was dusted with dry spores of *U. nigra*; lot 3 was inoculated with spores of *U. nigra* by means of a spore suspension in vacuum; lot 4 was inoculated the same as lot 3 and then treated with a 1:320 formaldehyde solution for 1 hour. Seeds of all 4 lots were sown in flats in a somewhat sandy soil adjusted to 50 per cent of its water-holding capacity. The flats were placed in 3 chambers automatically maintained at 5°, 15° and 30° C., respectively.

TABLE 2.—*Infection by loose smut (Ustilago nigra) in Alpha and Wisconsin Pedigree No. 5 barleys grown from seed artificially inoculated^a and sown in soil maintained at different temperatures and percentages of saturation until after the second leaf stage*

Series	Variety	Percentage soil saturation	Average number of plants grown ^b	Percentage plants infected at a soil temperature of:					
				5° C.	10° C.	15° C.	20° C.	25° C.	30° C.
I	Alpha	30	104	28	80	94	90	48	10
		47	107	13	91	93	86	85	3
		75	128	0	59	84	85	71	2
		Total or average	339	13	77	90	87	69	4
II	Alpha	35	106	73	85	92	97	79	19
		55	204	31	85	95	97	91	22
		90	16	7	32	22	58	56	0
		Total or average	326	41	78	88	92	84	19
III	Wisconsin Pedigree No. 5	35	73	59	60	81	79	52	4
		55	142	32	48	73	82	60	10
		90	22	0	50	71	65	47	0
		Total or average	237	40	52	75	80	53	7

^a The seed in Series I contained some natural inoculation.

^b At each combination of temperature and moisture.

The plants in the 30°, 15°, and 5° chambers emerged in 2, 6, and 28 days, respectively. They were kept in the different chambers under lights for several days after emergence, and then transferred to the greenhouse bench where they were grown to maturity. The data obtained are shown in table 3.

It is interesting to note that at 15° C. the higher percentage of infection resulted from inoculating the seed with dry spores and not, as might be expected, from applying a spore suspension in vacuum. This probably is due to the much heavier spore load put on the seed by the dry-spore method. The infection obtained after the seed had been treated indicates that it was naturally inoculated with *Ustilago nuda* to the extent of about 3 to 6 per cent. A comparison of these results with those from noninoculated, nontreated seed shows about the same amount of natural inoculation with *U. nigra*. The marked decrease in the amount of infection by *U. nigra* at 5° and 30°, compared with that at 15°, is outstanding.

In order to compare *Ustilago nigra* with *U. nuda* as to the effects of soil temperature before emergence upon the incidence of infection, seed of Alpha barley inoculated at flowering time with spores of *U. nuda* was included in the same experiment with seed of the same variety inoculated by dusting

TABLE 3.—Loose smut in Alpha barley grown to emergence at 5°, 15°, or 30° C. from seed naturally inoculated to an unknown extent with both loose smuts and either not inoculated further or inoculated artificially with spores of *U. nigra* by two different methods with or without subsequent treatment in formaldehyde

Inoculation and treatment	Data from plants grown to emergence at:								
	5° C.			15° C.			30° C.		
	Grown	Smutted		Grown	Smutted		Grown	Smutted	
	No.	No.	Per cent	No.	No.	Per cent	No.	No.	Per cent
a	150	9	6.0	150	21	13.9	140	14	10.0
b	647	31	4.8	621	559	90.0	614	113	18.4
c	758	68	9.0	670	585	87.3	590	107	18.1
d	125	5	4.0	120	7	5.8	111	3	2.7

a Not inoculated and not treated.

b Dusted with dry spores and not treated.

c Inoculated with a spore suspension under vacuum and not treated.

d Inoculated as in c and then treated in a 1: 320 formaldehyde solution for 1 hour.

with spores of *U. nigra*. The soil had a water-holding capacity of 37 per cent and was 50 per cent saturated. Six temperatures from 5 to 30° C. were used. Comparative results are shown in table 4. *U. nigra* caused a heavy infection—85.2 to 92.5—at temperatures from 10° to 25° C., inclusive, but produced only 12.6 and 2.6 per cent infection at 5° and 30°, respectively. *U. nuda*, however, produced the heaviest infection at 30° and almost as much at 5°. The data indicate that infection by *U. nuda* is not greatly affected by soil temperature during emergence, thus distinctly distinguishing it in this respect from *U. nigra*.

The possible effect of environmental factors after emergence on the incidence of smut has been suggested (4). To throw more light on this, seed of Alpha barley inoculated with spores of *Ustilago nigra* was sown in soil-temperature tanks maintained at 5°, 13°, and 30°, respectively. At emergence one third of each lot of plants was transferred to each of the other two temperatures, where the plants were grown until just before the heads appeared. The plants kept at 5° progressed so very slowly that it was found necessary finally to raise the temperature to 10° to bring about growth. This was done 3 months after planting. After the plants were almost in the boot they were kept at room temperature until smut data were taken, as shown in table 5.

It seems that the inhibiting effect of a soil temperature of 30° C. during emergence was not offset by a change after emergence to 13° C., a tem-

TABLE 4.—*Comparison of Ustilago nigra with U. nuda as to the influence of soil temperature before emergence on the incidence of infection in Alpha barley*

Soil temperature	Results from plants grown from seed inoculated with:					
	<i>Ustilago nigra</i>			<i>Ustilago nuda</i> ^a		
	Grown	Infected		Grown	Infected	
° C.	No.	No.	Per cent	No.	No.	Per cent
5	127	16	12.6	17	9	52.3
10	130	118	90.8	29	16	55.2
15	120	111	92.5	29	13	44.8
20	132	113	85.6	27	12	44.4
25	115	98	85.2	21	9	42.9
30	78	2	2.6	16	9	56.3

^a Inoculated in the flowering stage.

TABLE 5.—*Loose-smut infection in Alpha barley as affected by soil temperatures before and after emergence of the seedlings grown from seed inoculated with spores of Ustilago nigra*

Transfer number	Soil temperature		Infection results			Items compared	Odds
	Before emergence	After emergence	Total plants	Infected plants			
	° C.	° C.	No.	No.	Per cent		
1	30	30	338	16	4.7	1 and 2	1: 200
2	30	13	301	2	.7	1 and 3	1: 50
3	30	5 ^a	302	10	3.3	2 and 3	1: 430
4	13	30	307	91	29.6	4 and 5	Maximum
5	13	13	343	213	62.1	4 and 6	1: 640
6	13	5 ^a	318	144	45.3	5 and 6	1: 2000
7	5	30	324	128	42.6	7 and 8	1: 25
8	5	13	13	173	55.3	7 and 9	1: 100
9	5	5 ^a	308	96	31.3	8 and 9	Maximum

^a Later raised to 10° C. to induce growth and heading.

perature supposedly more favorable for smut development. Instead, there was a significant decrease in the percentage of infection, seemingly due to this change. No explanation is offered for this erratic result.

The transfers from 13° to both 30° and 5° seemed to yield more logical results, as both caused highly significant decreases in the percentages of infected plants.

Both lots of plants transferred from a soil temperature of 5° at emergence showed significantly greater percentages of smut than the lot remaining at 5°.

The growth of the latter plants was retarded and their development was abnormal. This was true also of the plants transferred to 5° from the two other temperatures. On the whole the results from the experiment, especially those obtained from an initial temperature of 13°, seem indicative of the probability that environmental conditions after emergence influence the development of smut caused by *Ustilago nigra*. It should be borne in mind that the above changes applied only to the roots of the plants. It is possible that environmental changes involving the entire plants would produce more striking results. Equipment for such a study was not available.

In taking infection data on the above experiments it was observed that those plants grown to emergence at a soil temperature of 5° or 10° very frequently produced sound heads on the main tillers, while the secondary tillers bore infected heads. This seemed to indicate that infection occurred after the plants had been transplanted to another soil temperature. It is generally accepted as fact that invasion of the barley seedling by *Ustilago hordei* does not occur after the first green leaf has pushed through the coleoptile. To determine whether or not this is true also of *U. nigra* the following experiment was carried out.

Seed of Alpha barley containing some natural inoculation and in addition inoculated artificially with spores of *Ustilago nigra* was sown in flats that were kept in constant-temperature chambers at 5° and 30° until after the first leaf had emerged from the coleoptile. The soil had a water-holding capacity of 30 per cent and was 50 per cent saturated. The seed was sown on March 1. Seed of this same lot, not artificially inoculated, was included in a parallel series. Simultaneously, some soil mixed with viable spores of *U. nigra* was stored in each chamber along with a quantity of spores in a glass dish. When the first leaf was through the coleoptile, the plants grown from artificially inoculated seed were divided into two lots. In lot 1 the seedling roots and stems were washed clean in running water and then the plants were transplanted to the greenhouse bench. In lot 2 they were transplanted without washing. The seedlings grown from noninoculated seed also were divided into two lots. In lot 3, before transplanting, the bases of the seedlings were blackened with spores kept in the same chamber in which the seedlings had been grown. In lot 4 the seedlings grown at 5° and 30° had packed about their bases spore-infested soil that had been kept at 5° and 30°, respectively, during the period of emergence. The seedlings from the 30° chamber were transplanted on March 8, while those from the 5° chamber were transplanted March 22. Unfortunately, on the night of March 13, 3 days before the seedlings emerged, the compressor failed and the temperature in the 5° chamber rose from 5° at 3 p. m. to 26° at 9 a. m. The flats were transferred to a 10° chamber until 2 p. m., when the 5° temperature was restored. Therefore, for 23 hours the temperature was above 5°. This most

probably accounts for the high percentage of infection in the plants grown from inoculated seed in the 5° chamber as shown in table 6.

TABLE 6.—*Infection with loose smuts in Alpha barley grown from artificially inoculated and noninoculated seed at initial temperatures of 5° C. and 30° C. and transferred in the first-leaf stage to the greenhouse bench (15° C. to 25° C.), half of the seedlings from inoculated seed being first washed thoroughly and those from noninoculated seed being either blackened with spores or transferred to spore-infected soil*

Conditions at planting		Conditions at transplanting	Infection results		
Temperature	Artificial inoculation		Total plants	Plants smutted	
° C.			No.	No.	Per cent
5	spores	a	178	151	84.8
5	spores	b	185	113	61.2
5	none	c	157	7	4.5
5	none	d	158	7	4.4
30	spores	a	186	5	2.7
30	spores	b	266	7	2.6
30	none	c	135	0	0.0
30	none	d	136	2	1.5
15-25	spores	not transplanted	80	56	70.0
15-25	none	not transplanted	79	4	5.0

a Seedlings thoroughly washed.

b Seedlings not washed.

c Seedlings dipped in spores.

d Seedlings transplanted to spore-infested soil.

Instead of depressing the percentage of infection, washing the seedlings at transplanting increased it consistently and significantly, compared with unwashed seedlings. That no invasion of the seedlings took place after the first leaf had emerged, that is, after transplanting, is indicated by the low percentage of heads smutted when the seed was not inoculated. Most of these heads, in color and shape, were typical of infection by *Ustilago nuda*, especially those grown to emergence at 30°.

Effect of Soil Composition

The experiments involving different kinds of soil yielded mostly negative results (Table 7). In series I the percentage of infected plants grown in "garden soil" seemed significantly less than that of plants grown in sand or Keyport silt loam, but this significant difference was not duplicated in series II, although the average per cent of infection here also was slightly lower. An analysis of the detailed data from the experiment as a whole indicates that infection is not greatly influenced by soil composition.

TABLE 7.—*Loose smut infection in Alpha barley grown from seed artificially inoculated with spores of Ustilago nigra and sown in three kinds of soil—(a) river sand, (b) Keyport silt loam and (c) black "garden soil"*

Series	Soil	Plants			Heads		
		Grown	Infected		Grown	Infected	
		No.	No.	Per cent	No.	No.	Per cent
I	Sand	449	325	72.4	564	405	71.8
	Loam	473	315	66.6	570	379	66.5
	"Garden soil"	397	193	48.6	557	244	43.8
II	Sand	235	219	93.2	551	513	93.1
	Loam	169	157	92.9	576	539	93.6
	"Garden soil"	204	182	89.2	593	502	84.7

Soil Reaction

In the first experiment in which soil reaction was the variable, a significantly higher percentage of smutted plants developed in the acid soil than in the basic soil (Table 8). Differences between results obtained in neutral and acid soils and neutral and basic soils seemed of no significance statistically.

In the second experiment the results were the reverse of those obtained in the first one. A significantly lower percentage of smutted plants developed in the extremely acid soil than in either neutral or basic soil. Differences in results in the two latter soils were not significant. It is possible, however, that had many of the plants that failed to survive in the extremely acid soil been brought to maturity the results would have been different. It is probable that most of the plants that died were those previously weakened by smut invasion.

In the third experiment the acid soil of the second experiment had its pH raised from 3.8 to 4.7 by the addition of lime, and another planting was made later in this modified soil, as well as in soil of pH 7.7. As in the previous experiments, 8 replications were used. The differences in the results obtained on the 2 soils were not significant. The above 3 experiments as a whole failed to show any consistent effect of soil reaction on infection. It is evident that *Ustilago nigra* can develop in plants grown in soil that is beyond the acid tolerance of barley. In studies involving breeding for resistance or seed treatments soil reaction, therefore, is not likely to be an important factor.

DISCUSSION

In general, the reaction of *Ustilago nigra* to soil temperature in the infection of barley appears to be similar to that of *U. hordei* as determined by

TABLE 8.—*Loose smut infection in Alpha barley grown from seed artificially inoculated with spores of Ustilago nigra and sown in soil adjusted to different degrees of acidity or alkalinity*

Experiment number	Soil reaction	Plants			Heads		
		Grown	Infected		Grown	Infected	
	<i>pH</i>	<i>No.</i>	<i>No.</i>	<i>Per cent</i>	<i>No.</i>	<i>No.</i>	<i>Per cent</i>
Ia	4.2	275	173	62.9	300	180	60.0
	7.0	363	189	52.1	424	205	48.3
	8.0	409	178	43.5	491	192	39.1
IIb	3.8	127	51	40.2	532	183	34.4
	6.9	206	191	92.7	677	592	87.4
	8.1	215	189	87.9	677	574	84.8
III	4.7	243	211	86.8	453	397	87.6
	7.7	285	231	81.1	689	520	75.5

^a Carried out in flats. If unheaded plants, omitted in calculations, were counted as noninfected the resulting percentages would be 42, 40, and 44 for acid, neutral and basic soils, respectively.

^b In this experiment the plants had to be transferred from the acid soil to other soil to make continued growth possible. Many plants failed to develop.

Faris (1); that is, relatively low percentages of infection at 5° and 30° and fairly high percentages from 10° to 25°, with the optimum around 15°. The optimum temperature is somewhat higher than that for infection by bunt as determined by several investigators, and a trifle lower than that for the smuts of oats (5). As in the case of some of the other cereal smuts, the percentage of infection by *U. nigra*, under certain conditions, is depressed by excessive soil moisture during the period of emergence. This, undoubtedly, is due to oxygen deficiency. On the other hand, *U. nigra* seems to show no consistent response to differences in soil reaction in its infection percentages, while the covered smuts of barley and oats do (4, 5). It is probable that in experiments by various investigators reported prior to Tapke's work, the barley "loose smut," shown to be affected by environmental conditions, was *U. nigra*. Tapke (7) has demonstrated the reasonableness of this assumption.

SUMMARY

Soil with a high percentage of saturation proved generally unfavorable for infection by *Ustilago nigra*, especially at 5° and 30°, while relatively dry soil favored infection at 5°. At the other temperatures differences in soil moisture from 30 to 55 per cent saturation did not appear to influence infection.

The cardinal temperatures for infection by *Ustilago nigra* were found to be: Minimum below 5°, optimum 15° to 20°, and maximum above 30° C. Fairly high percentages of infection were secured at soil temperatures of 10°, 15°, 20°, and 25° C. *U. nuda*, on the other hand, showed little if any significant reaction to temperature.

Plants grown to emergence at 30° and then changed to a soil temperature of 13° C. showed less infection than those kept at a soil temperature of 30° C. until near heading. However, plants transferred at emergence from a soil temperature of 13° to 30°, or 5° showed a highly significant decrease in the percentage of infection compared with those kept at 13°. Likewise, those similarly transferred at emergence from 5° to 13° and 30° showed significant increases in the percentages of infection, compared with those kept longer at the lower soil temperature.

Dusting the seed with dry spores of *Ustilago nigra* resulted in heavier infection than inoculating the seed by means of a spore suspension in vacuum.

Results from experiments, in which emerging barley seedlings were inoculated with spores and with spore-infested soil, indicate that infection by *Ustilago nigra* does not occur after the first leaf has emerged.

BUREAU OF PLANT INDUSTRY,

U. S. DEPARTMENT OF AGRICULTURE.

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RELATION OF NITROGEN-CARBOHYDRATE NUTRITION OF STAYMAN APPLE TREES TO SUSCEPTIBILITY TO FIRE BLIGHT¹

GEORGE K. K. LINK AND H. W. WILCOX

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This paper reports a study of the relation of succulence and hardness of apple shoots to infection by *Erwinia amylovora*. Differences in metabolic status with resultant differences in chemical and structural constitution, and in disposition to infection were induced by application and nonapplication of $\text{Ca}(\text{NO}_3)_2$.

Horticulturists and pathologists observed early that application of nitrogenous manures enhanced the susceptibility of pear and apple trees to spontaneous and experimental fire blight. Orchard experiments corroborated these observations (23, 28, p. 467, 29). Recently, in a study of environal factors disposing apples to fire blight, Shaw (25) made a few preliminary pot experiments with partial control of soil nutrients. Shoots grown in "sand-soil" blighted more severely than those grown in sand with sodium nitrate added. It was concluded that soil nutrients had a distinct effect on the susceptibility of Northwest Greening shoots and slight effect on the susceptibility of Wealthy shoots.

Experiments here reported involve more complete control of nutrition than any yet reported for potted trees inoculated with *Erwinia amylovora*. The reader interested in a bibliography is referred to recent publications on fire blight that give extensive citations of earlier papers (14, 22, 24, 25, 34).

MATERIALS AND EXPERIMENTAL PROCEDURE

The trees, root grafts of the Stayman variety, received from a commercial nursery in the fall of 1933 by G. T. Nightingale, were placed at the writer's disposal April 1, 1934 (18). On December 1, 1933, after several weeks in cold storage, the roots were thoroughly washed and pruned, leaving only old roots 0.5 in. or more in diameter. The tops also were pruned, leaving several buds, and an axis approximately 15 inches long.

The trees were then planted 4 per pot in the 2-gallon percolator urns used in the Chicago soil nutrient-temperature tanks (12). After covering the drain holes with glass wool, the pots were nearly filled with white nitrogen-free quartz sand, fine enough to pass a 1.0 mm. but not a 0.5 mm. sieve.

The trees were stored 2 months at 35–38° F., meantime receiving weekly applications of minus NO_3 (–N) solution (Table 1) sufficient to flush the

¹ This study was supported in part by a grant from the Rockefeller Foundation to the University of Chicago.

TABLE 1.—Composition of nutrient solutions^a (partial volume molecular concentration of salts used)

	Ca(NO ₃) ₂	KH ₂ PO ₄	MgSO ₄	CaCl ₂
Complete, plus NO ₃ (+N)	0.0090	0.0045	0.0045
minus NO ₃ (-N)	0.0045	0.0045	0.0090

^a The pH value of these solutions was adjusted to 4.5 by addition of conc. H₂SO₄.

substrate. Distilled water was added as needed to keep the substrate moist.

On January 31, 1934, the trees were moved to a greenhouse, where the temperature was regulated to approximately 65–70° F. during the day and 60–65° F. at night. Daily applications of –NO₃ solution sufficient to flush the sand were made. Weekly, each pot was washed with several liters of distilled water, after which –NO₃ nutrient solution was applied immediately. All pots received two applications of +NO₃ nutrient solution between January 31 and February 15.

After bud development commenced, all but one bud per tree were removed from one lot of plants (8 pots, 32 trees) and all but 2 buds per tree were removed from the other lot (7 pots, 28 trees).

On April 10, 1934, all pots were transferred to tanks in another greenhouse. No attempt was made to control air and soil temperature, or humidity. During April, day temperature was 65–70° F.; night temperature, 60–65° F. During May, June, and July on dry, sunny days the temperature rose to 120° F. At no time, however, did the trees manifest water shortage.

On April 13, 1934, it was apparent that the shoots were about to develop terminal buds and cease elongating. Consequently, 750 cc. per pot of +NO₃ nutrient solution was applied to the 8-pot group of trees, hereafter designated as the +N lot. (Table 1). Each pot of the 7-pot lot received 750 cc. of the –NO₃ solution (Table 1), hereafter designated as the –N lot. Following this treatment both lots were watered daily with acidulated tap water. There was prompt response in the +N lot to the single nitrate application. The leaves became greener in 24 hours. By the 27th of April, 1934, striking color and form differences were noticeable in the shoots of the +N and –N lots.

From April 27 to May 2 all but one shoot of both the +N and –N lots were inoculated² with a 24-hour culture of *Erwinia amylovora*³ in nutrient broth by injection with a hypodermic syringe. Each shoot was inoculated in the middle of the second apical internode and in the basal internode.

² The writers were assisted in the original inoculation by Messrs. W. S. Cook, W. S. Phillips, and Miss Frances Jewett.

³ The culture was obtained from Dr. H. R. Rosen.

The shoots, especially tips, of the +N lot were succulent and readily inoculated; those of the -N lot were woody and inoculated with difficulty.

In the +N group, after blighting of the tip, one or more lateral shoots developed from the new wood. These were inoculated June 6. Following death of their tips was similar development of new lateral shoots from the first lateral shoot. These were inoculated July 13.

On June 11 half the trees of each lot were given an additional application of +NO₃ solution. In the -N lot this treatment, with shading, was an attempt to break dormancy and to initiate enlargement of the apical and basal cankers. In the +N lot this treatment was applied to determine whether increased diameter growth would lead to girdling of the axis by the basal cankers.

After August 1 the pots were kept moist with nitrate-free tap water with occasional application of -NO₃ solution, left in a cool greenhouse until January 1, 1935, and then transferred to cold storage.

Late in March, 1935, the trees were transferred to the greenhouse as in 1934. This time, however, the +N and the -N lots received biweekly applications of 750 cc. of the +NO₃ and -NO₃ solutions, respectively, and daily applications of acidulated tap water (pH 4.5), with occasional thorough flushing of the pots, to lower the concentration of the accumulating salts.

On April 22, after trees of both lots had developed succulent shoots, the apical internodes of some of them were inoculated.

Thereafter, during April, May, June, and July, each pot of the +N and -N lots received, daily, 500 cc. of +NO₃ and -NO₃ solution, respectively. By June 7, shoots of the -N lot had ceased elongation. Shoots of both lots were inoculated in the second apical internode. Those of the -N lot were placed in sunshine to further carbohydrate synthesis and hardening, while those of the +N lot were shaded part of the day. Contrasting uncontrolled weather factors in these experiments during spring and summer of the 2 years of 1934 and 1935, there were fewer sunny days, average and maximum temperatures were lower, and humidity was higher in 1935 than in 1934.

In 1934, both control and inoculated shoots were cut off, photographed, and histological examinations made after fixing them in Flemming's weaker fixative and embedding in paraffin. In 1935, shoots of each lot were wounded and fixed at definite intervals in order to determine rates of wound-periderm formation. The results of these experiments will be reported elsewhere.

RESULTS

At the time of first inoculation in 1934, shoots of the -N lot of plants had ceased elongating, developed terminal buds, were hard throughout their

entire length, and highly colored with anthocyanin. Leaves were hard and yellowish green. The average length was 6.7 inches and the average number of nodes was 9.9 (Table 2).

Microscopic examination of sections of the -N axis revealed an abundance of starch in the pith, xylem, rays, pericycle, and inner cortex. The middle and lower internodes showed more starch, more thick-wall parenchymatous and sclerenchymatous elements, and more accompanying atrophy of protoplasts than the upper internodes.*

Following the 1934 inoculations, none of the -N shoots developed leaf-killing or stem-girdling lesions in either the top or basal internodes. Apical lesions were small after 48 days, averaging less than $\frac{1}{4}$ inch. Basal lesions were even smaller.

The single application of $+NO_3$ to half the trees in the -N lot led to some diameter increase and elongation of internodes, but did not break their dormancy. Tip and basal lesions increased slightly. In one instance the tip lesion became $\frac{3}{4}$ in. long, but without killing leaves or girdling the axis.

Following inoculation in 1935, 3 shoots in this lot developed apical lesions $\frac{1}{4}$ to $\frac{3}{4}$ in. long. Other than these lesions, all trees in the -N group reacted the same in the 1935 as in the 1934 experiments.

At the time of the first inoculation in 1934, the +N shoots were still elongating with no terminal buds. Upper internodes were very soft but lower ones had begun to harden and develop anthocyanin. The leaves, especially the upper ones, were large, tender, and dark green. Shoots averaged 16 inches in length and 14 internodes.

Contrasted with corresponding sections of the -N axis, the +N tissue showed less starch, less thick-wall parenchyma, fewer sclerenchymatous elements, and fewer atrophied protoplasts. The +N lower internodes resembled -N upper internodes.

Following apical inoculation, an average of 33 per cent of the average length of +N shoots had blighted at the time the lesions became inactive. Maximum killing involved 45 per cent of the shoot. Final readings (Table 2) were made after 46 days. By this time many lesions had been inactive at least 16 days.

Lateral shoots that developed after death of the original shoot tip averaged 10 inches in length, with 9 internodes. Following apical inoculation

* The gradual disappearance of the protoplast in maturing phellem, fibers, stone cells, tracheids, and vessel elements may be considered atrophy of the protoplast, that is, of the cell proper, even though accompanied by wall hypertrophy. Unfortunately the term cell is applied indiscriminately to the protoplast, to the protoplast plus its wall, and to the wall plus the space once occupied by the protoplast. Atrophy of the protoplast is a permissible description.

TABLE 2.—*Inoculation experiments with shoots of Stayman apple trees of differing succulence and hardness following application and non-application of $\text{Ca}(\text{NO}_3)_2$*

Year	Status of shoot at time of inoculation	Date of inoculation	No. of trees	No. of shoots	Length of shoot in inches at time of inoculation		Size of lesions after 46 days			
					Max.	Average	Non-girdling non-leaf-killing in 1/16 in.		Girdling leaf-killing in per cent of length of shoots	
							Tip	Base	Tip	Average
1934	-N; terminal bud formed	4/28-5/2	28	55	18	6.7	3 × 2	2 × 1.5	0	0
	+N; no terminal bud formed (1)	4/28-5/2	32	31	25	14		5 × 3	45	33
	Lateral from blighted shoot (1); no terminal bud formed (1a)	6/6	32	31	15	10			100	25
	Lateral from blighted lateral (1a); no terminal bud formed	7/13	32	31	10	3.3			30	18
1934	1935									
-N	-N; no terminal bud formed	4/22	3	3	4	3				100
+N	+N; no terminal bud formed	4/22	3	3	6	5				100
-N	-N; terminal bud formed	6/7	15	15	10	6.6	3 × 2		0	0
+N	+N; no terminal bud formed	6/7	16	16 ^a	33	21			90 ^b	59 ^b

^a 1 failure in a 12-inch shoot.^b Still progressing after 48 days.

23 per cent of their average length blighted, and, in a few cases, the entire shoot. However, such lesions did not penetrate the older wood.

Similarly, lateral shoots developed from the above blighted lateral shoots. These averaged 3.3 inches in length with 6.7 nodes. After apical inoculation 18 per cent of the average length of these shoots was blighted.

Basal lesions of the $-N$ shoots averaged $3/16 \times 5/16$ in. Two involved $\frac{1}{2}$ to $\frac{3}{4}$ the circumference of the stem, but none girdled the axis or killed leaves.

Following the additional application of $+NO_3$ solution to half the $+N$ lot, the basal lesions increased with the increased axis diameter and attained an average size of $5/16 \times 7/16$ in. Two induced local hypertrophy equal to 10 per cent of the diameter of the axis without, however, leading to other fire blight symptoms. Dormancy in the central shoot was not broken by this treatment.

The 1935 experiments corroborated those of 1934, showing even more striking contrast between the $-N$ and $+N$ shoots. This was partly due to the increased NO_3 application and partial shading of the $+N$ plants and partly to the lower average temperature, greater cloudiness, and higher humidity—factors all favoring succulent development.

Thus, in 1934, the noninoculated $-N$ control shoot ceased elongating by May 25, after attaining a length of 18 inches; the equivalent $+N$ shoot ceased by June 11, after attaining a length of 25 inches. In 1935 $-N$ control shoots stopped elongating June 6 at a length of 20 inches, while control $+N$ shoots were still elongating July 25, they having attained at that time a maximum length of 34 inches with 36 nodes.

In 1934, tip lesions in inoculated $+N$ shoots had ceased activity by June 11, that is, after 46 days, though most of them ceased after 30 days. In 1935, similar tip lesions were still advancing on July 25, 48 days after inoculation—an average of 59 per cent of the shoot length having been killed. Maximum killing was 27 inches, or 90 per cent of the shoot.

Inoculation on April 22, 1935, of a few very young actively growing shoots of both $-N$ and $+N$ lots, resulted in killing the entire shoot back to the old wood. The average length of the $+N$ shoots was 5 inches; that of the $-N$ shoots, 3 inches.

Histological study of inoculated shoots revealed very limited bacterial invasion in the tip and basal lesions of the $-N$ lot and in basal lesions of the $+N$ lot. In all cases the lesion was well walled off with wound periderm. The parasite was found in all tissues of the axis. Its location in the tissues and gross injuries to the middle lamella, primary and secondary walls, and protoplasts of succulent tissues is essentially that noted by other investigators (7, 14, 21, 24, 25).

DISCUSSION

Succulence and Disposition⁵

The experimental results indicate that disposition of Stayman apple shoots to fire blight is correlated with maturity of the inoculated part (14, 23, 24, 25, 29). If, at the time of inoculation, inoculated and adjacent tissues have ceased cell division and enlargement and have matured into thick-wall constituents, the relatively woody⁶ shoot is negatively disposed to infection by *Erwinia amylovora* and, therefore, is considered resistant. If, on the other hand, there is cell division and enlargement and little maturation of tissues, the relatively soft succulent shoot is positively disposed to infection and is considered susceptible. Behavior of all lesions on non-elongating shoots and basal lesions in +N shoots corroborate the former statement, apical lesions in elongating shoots the latter. These results harmonize with earlier reports on other varieties (23, 29). The most susceptible varieties apparently produce and maintain the greatest proportion of succulent thin-wall tissues abounding in living protoplasts.

In the 1934 experiments, tip lesions in the +N lot did not progress far down the stem; the maximum amount of shoot blighted was 45 per cent; the average, 35 per cent. Most lesions on the main axis were inactive after 30 days; those on the lateral axis, after 15 days.

During the 1935 experiments additional nitrate applications, partial shading, lower temperatures, and higher humidity favored greater succulence. Under these conditions a maximum of 90 per cent and average of 59 per cent of the shoot was killed. After 48 days the lesion was still progressing into the apparently hard axis.

These observations indicate that, under favorable conditions, as the blighting advances down the axis, it progressively reactivates meristematic tissue in the matured hard parts of the axis. The cambium again gives rise to succulent thin-wall cells, and protoplasts of the heavy-wall cortical, pericyclic, phloem, xylem, pith, and ray parenchymas become active. Enzymatic activity thins the walls, and dividing cells give rise to new thin walls within the old ones. Regions, once poor in active protoplasts and thin walls, become relatively rich in both. Thus, progressive invasion by the parasite comes with increased succulence in the formerly hard, lower internodes of the axis. Similarly activated parenchymatous cells of the leaf and branch

⁵ By disposition of the apple shoot and of *Erwinia amylovora* is meant that aspect of their constitutions rendering them either prepared or unprepared for infection.

⁶ The terms woody and succulent are not used here solely as chemical concepts to designate the presence of lignin and of water respectively. Lüdtke (13) has pointed out that lignified or woody designates a total habit or status of particular tissues, organs, or plants, describing their morphological, physiological, ecological, and teleological properties. The same is true of the term succulent.

gaps are involved and, through them, petioles and branches are invaded. The susceptibility of the tree seems to depend upon the initial amount of succulent tissue formed during the main growth period, retention of succulent tissue as the axis matures, and resumption of meristematic activity by relatively inactive protoplasts.

These data indicate that the regulation of relative carbohydrate and nitrogen nutrition may be used experimentally to induce expression of succulence and susceptibility or woodiness and insusceptibility (11). Given identical environmental conditions, the metabolic status of trees supplied with the nutrient solution containing nitrate can be kept at a level favoring meristematic activity, cell enlargement, succulence, and susceptibility. By withholding or washing out nitrates, that metabolic level can be so changed that these come to an abrupt end (3, 4, 5). Shading or nonshading, etc., produce similar effects. The resulting +N or succulent plants are relatively low in carbohydrate and high in nitrate-free nitrogen compounds; the converse is true of the -N or woody plants (8, 19, 20, 26).⁷

In +N plants the balance of carbohydrate and nitrogen metabolisms, enzyme and hormone actions favors organic nitrogen and protoplasm synthesis (8, 19, 20, 26) with resultant cell division and enlargement. Protoplastic atrophy is delayed and infrequent; cell walls are thin; relatively few plasma-insoluble polysaccharides and nitrogenous compounds accumulate. The protoplasts are poor in starch, but rich in plasma-soluble organic nitrogen compounds. According to Lüdtke (13), young or thickening cell walls, in addition to pectic substances, cellulose, hemicellulose and soluble lignin and lignin-like compounds, contain plasma-soluble polysaccharides, amyloids, and intercelluloses that become converted into cellulose. Similarly, probable conversions accomplished in young walls are: interxylans to xylans, plasma-soluble intermediary stages of galactans and arabans to plasma-insoluble forms; and the final synthesis of pectins from linkages of polygalacturonic acids, galactose, and arabinose. If these interpretations are correct, primary and secondary walls and middle lamellae in course of formation contain enzymes that accomplish such synthesis and condensation, yielding end products characteristic of those structures.

In -N shoots carbohydrate and nitrogen metabolisms, enzyme and hormone action result in diminished synthesis of organic nitrogen and protoplasm (8, 19, 20, 26) and consequent checking of cell division, thickening of cell walls, and protoplastic atrophy. Plasma-insoluble polymeric carbohydrates and plasma-insoluble nitrogen compounds accumulate. Protoplasts are rich in starch, poor in plastic organic nitrogen compounds (16, 17). In the course of considerable secondary change constituents of the

⁷ These publications, especially 8 and 26, contain extensive bibliographies on carbohydrate-nitrogen nutrition of apple trees.

middle lamellae and primary walls become less hydrated and less soluble. They contain, therefore, comparatively few pectic substances and a high proportion of the polymeric polysaccharides known as hemicelluloses (6, 15, 16), some cellulose (26), and the less soluble lignin (13, 26), and lignin-like compounds (13).

Other metabolic differences in +N and -N shoots exist, since the former are probably richer in auxins and cell-division-stimulation substances than the latter (35). The pH values also may differ because of altered proportions of tissues predominantly acid or alkaline (17). Environmental factors, such as air temperature (14, 19, 25), humidity (14, 20, 25), soil temperature (18, 25), and soil moisture (25), may be limiting in altering metabolic status as expressed through altered meristematic activity of genotypically identical plants. Thus, these factors influence relative rate of wound periderm formation (20, 25) and also disposition of apples to infection by *Erwinia amylovora* (25).

The query arises: what host characteristics favor infection? Hard, woody tissues of genotypically susceptible varieties and succulent tissues of genotypically insusceptible varieties may contain substances that inhibit the parasite. Examinations of punctures in such shoots show that introduced organisms do not increase appreciably before the wound is healed. Fewer bacteria and less gum are formed during the period required for wound periderm formation than during an equivalent period in a favorable site. Usually a band of apparently unaffected cells lies between the advancing edge of the bacterial mass and the periderm (25), indicating that the bacteria are first stopped, and then periderm formation follows. Its formation appears to be favored by conditions unfavorable to the parasite, and is more a regenerative (25) than it is the primary defensive reaction it has been interpreted to be (14).

The presence of abundant water in the intracellular spaces of succulent tissues favors invasion in providing a physical and nutritive medium for bacterial extension. However, if water were the limiting factor, suffused tissues of any plant should be susceptible to invasion by *Erwinia amylovora*. It seems probable that certain physical and chemical characteristics, such as the presence of soluble carbohydrates and nitrogen compounds and host hormones concomitant with wateriness of tissues favor propagation of bacterial thalli, as well as host cells.

Causal Complex of Metabolic Status and Disposition

The metabolic status of a shoot prior to and at time of inoculation is important in disposing it to infection. This status is conditioned by a causal complex of internal and external factors (9, 10, 11). The internal factors include the hereditary constitution and past experience of the shoot, and

correlative influence of other parts of the plant. Heredity determines the range of metabolic possibilities. Stayman is neither as susceptible or insusceptible as other apple varieties (24). At the time of inoculation certain internodes and tissues were conditioned not only by existing temperature, humidity, and other environmental factors, but by previously induced effects of these. The single application of nitrates to certain trees in 1934 produced effects evident not only in 1934, but in 1935. The past-experience factor is the result of all internal and external past factors that affected a given part prior to a given moment.

Correlative influences active at the time of inoculation are evident in the effect of root tips on the inoculated shoot. The root tips absorb the available nitrates, reduce them to nitrites and ammonium compounds, oxidize carbohydrates and their derivatives, synthesize amino acids, polypeptids, and transportable proteins, and thus affect the metabolism and disposition of the shoot (3, 4, 5, 19, 20, 26, 31).

After inoculation, blighting of a shoot generally led to development of a susceptible shoot from a lateral bud when the remainder of the plant was phenotypically insusceptible. Blasting of the terminal bud led to removal of its inhibitory influence through hormones upon the development of lateral shoots (2, 27, 30, 35). At the moment of inoculation the influence of this correlative factor has become part of the past experience of the inoculated shoot.

In these experiments, prior to its use as inoculum, the disposition of the parasite was considered a constant. Thereafter environmental changes must have significantly affected the organism when inoculated into tissues of such differing constitution as those in - N and + N shoots.

Before conclusions can be drawn relative to susceptibility to *Erwinia amylovora*, the particular plant must be grown under conditions favoring maximum vegetative growth and succulence. These factors for the apple are: application of nitrogen, limited light exposure, high humidity, moderate air and soil temperature, high soil moisture, low concentration of mineral nutrients, and a soil pH of approximately 4.5 (18, 20, 32, 33). In these experiments the potential susceptibility of the Stayman variety may not have come to full expression, since some factors influencing the trees were not conducive to metabolism that leads to maximum meristematic activity and accompanying succulence.

The Course of Injury in Infected Tissues

There is no agreement among recent competent investigators as to the nature of primary injury, mechanisms inducing it, and the later course of pathic events in the tissues (7, 14, 21, 24, 25).

Punctured succulent tissues suffer a rupture of walls and protoplasts, and from shock of adjacent unpenetrated cells; integrative properties of the

healthy protoplast are lost; and enzyme systems through unregulated catalysis often damage host tissues further and produce changes favoring invasion. In certain rosaceous plants, especially the stone fruits, there is a tendency toward progressive self-injury with copious gum formation following even simple mechanical wounds. Disruption of lignin and lignin-like substances in which the polymeric carbohydrates of walls are embedded (13) expose them to digestive and oxidative changes. Altered permeability as well as broken cell membranes make the wound abound with protoplasmic contents. Thus a favorable medium is provided for the parasite in potentially susceptible hosts. Unless trauma leads to release of substances inhibiting the infective agent, a wound in succulent tissue provides better ingress than one in woody dehydrated tissue. In the course of these experiments it was found that in succulent tissues the wound was still filled with fluid 15 minutes after inoculation, while that in woody tissues was dry.

In non-wounded tissue the parasite must find or create self-favoring conditions. It is known that *Erwinia amylovora* is able to enter through the unbroken surfaces of nectaries or natural openings and establish itself in initially intact tissues. Whether the parasite *per se* possesses capacities to bring about directly all the destruction of middle lamellae, walls, and protoplasts noted in infected tissues, is not known. It may inflict a primary minor physical injury as a result of which cell processes become unregulated, initiate autolysis, and bring about the course of pathic events characteristic of tissue invaded by *Erwinia amylovora*. The gummy mass in which the bacterial thalli are embedded might be derived entirely from disturbed cells, and inhibit their prompt, healthy functioning. This together with imbibitional and osmotic forces (14) of the thalli might further injure the protoplast, alter its permeability, and account for the plasmolysis noted as early evidence of injury in infected tissues. Complete enclosure of cells (21, 7) in this mass and resultant interference with free influx and outflow of metabolic materials and by-products (24) leads to their injury and death. Dissolution of middle lamellae and plasmodesmata preliminary to such engulfment may be due entirely to enzymatic action of the disturbed wall and protoplast, though from observations of infected tissue (7, 14, 21, 24, 25) it seems more likely that the parasite physically or preferably chemically initiates such disorganization. Miller (14) reports that nutrient medium in which *Erwinia amylovora* was grown contained in low concentrations hydrolyzing enzymes that destroy the middle lamella of carrot. This capacity alone or in conjunction with heightened activity of enzyme systems in the injured protoplast and wall would further augment existing damage.

Whether nonregulated enzyme systems of the host, or enzymatic and mechanical destructive forces of the parasite, or both, play the critical rôle in initial host injury, the metabolic and structural status of host tissue

is important in conditioning phenotypic expression of genotypically determined capacity to develop susceptibility or resistance.

CONCLUSIONS

1. Shoots of Stayman apple trees grown in quartz sand were rendered succulent and woody, respectively, through the application of either plus or minus nitrate nutrient solutions, and inoculated with *Erwinia amylovora*.

2. Actively growing succulent shoots were susceptible. Shoots in which tissues had matured to the extent that they could not be restored to meristematic activity were not susceptible.

3. Application of nitrates with particular light, temperature, and moisture conditions favored a metabolic status resulting in succulence and susceptibility to infection of Stayman apple shoots by *Erwinia amylovora*. Nonapplication of nitrates under the same conditions favored early cessation of meristematic activity, resulting in woodiness and resistance to infection.

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THE DISTRIBUTION, CAUSE, AND RELATIVE IMPORTANCE OF CRANBERRY FRUIT ROTS IN MASSACHUSETTS IN 1932 AND 1933, AND THEIR CONTROL BY SPRAYING

HERBERT F. BERGMAN AND MARGUERITE S. WILCOX

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The control of cranberry fruit rots in Massachusetts and New Jersey is a problem of primary commercial importance. Shear (4), in 1920, estimated that the annual loss from fruit rot averaged at least 25 per cent of the total cranberry crop of the United States. This estimate was reaffirmed by Shear, Stevens, and Bain (5) in 1931. Studies on various phases of the problem of the control of fruit rots have been carried on for some 30 years. Material progress has been made, but much yet remains to be done. A recapitulation of the results up to 1931 is given in the publication just cited.

As a part of spraying and storage experiments carried on in 1932 and 1933, studies have been made on the distribution and relative importance on certain bogs, of fungi causing fruit rots of cranberries in Massachusetts, and on the degree of control of specific fungi by spraying. The bogs on which spray plots were located are widely separated and represent considerable diversity in local conditions. The bogs are designated as No. 1, 2, 3 in this article, S.E. and N.E. refer to plot location. The varieties on these bogs are mainly Early Black and Howes, the sorts most extensively grown in Massachusetts. Two minor varieties, Middleboro and Holliston, also are included.

In these experiments duplicate plots were laid out that received 1, 2, and 3 applications of spray material, respectively, during the season, with adequate check plots. The results are given for specific individual plots. All plots had an area of 1/20 acre, except those on bog 1, which were 1/50 acre. A Bordeaux mixture made up with granular CuSO_4 and chemically hydrated lime, using a 4-6-50 formula with one pound of potassium fish-oil soap for each 50 gallons of water, was applied to most of the plots. Some mercurial fungicides were used on a few plots in 1932. A small experimental power sprayer was employed, capable of producing a maximum pressure of 100 pounds per square inch at the pump, when spraying. The pressure at the nozzle was probably not more than 75-80 pounds. The spray was applied at the rate of 300-400 gallons per acre at each application. The first application was made when the first flowers were just beginning to open. When only one application per season was given it was at this time. A second application was made as soon as the petals had fallen from most of the flowers. A third application was made about the 10th to the 15th of August or about three weeks after the second. The mercurial sprays were prepared

by dissolving one pound of the material in 100 gallons of water. The time, rate, and method of application were the same as for Bordeaux.

The percentage of total spoilage due to a particular fungus on a given plot was determined by a method essentially the same as that described by Stevens and Bain (6, p. 650). In calculating the results the modification suggested by them (7, p. 809) was used. In this work a quarter-barrel stock sample was made up as the berries were harvested by taking one to three quarts of berries from each picking box belonging to a particular plot. These stock samples were stored in the basement of the Cranberry Experiment Station at East Wareham, Massachusetts, for use in subsequent tests.

The first determination of spoilage of berries from all plots was made usually within 1 to 2 days and except in a single case, as stated below, never later than a week after the berries had been picked. Four-quart subsamples from each stock box generally were used and the percentage of spoilage was determined by weight. In September, 1932, the determination was made by count from 3-pint samples and in September, 1933, by weight from 1-quart samples. The percentage of rot present at harvest was not determined immediately for berries picked after October 15, 1932, the berries being allowed to stand until mid-November before sampling.

The causes of subsequent spoilage in the various lots were determined by the following procedure. About the middle of each month, from October to January, inclusive, a 4-quart subsample of sound berries was taken from each quarter-barrel stock sample and stored for 2 weeks in the basement, under the same conditions as the samples from which they were taken. Then the subsamples were carefully sorted and the percentage of spoiled berries determined. During the winter of 1933-34 the percentage of rot present in the quarter-barrel stock samples was determined at the middle of each month, when the subsamples for storage tests were sorted out. Cultures were made from spoiled berries from representative lots, when the subsamples were sorted out at the time of picking and also at the end of each 2-week storage test. The cultures were held at room temperature until the fungi fruited or developed sufficiently for identification. In this way the progress of spoilage in berries due to any specific fungus could be followed from the time the berries were picked until the end of the storage season on February 1. In 1932-33 determinations of the amount of rot present in stock samples at the middle of each month were not made, but the berries from the plots on one bog were screened the middle of October and from the other bog about the middle of November and the percentage of spoiled berries removed during screening was substituted for these values. For these two bogs, therefore, the progress of spoilage during the season can be followed and compared with that in 1933. This comparison is shown graphically in figure 1.

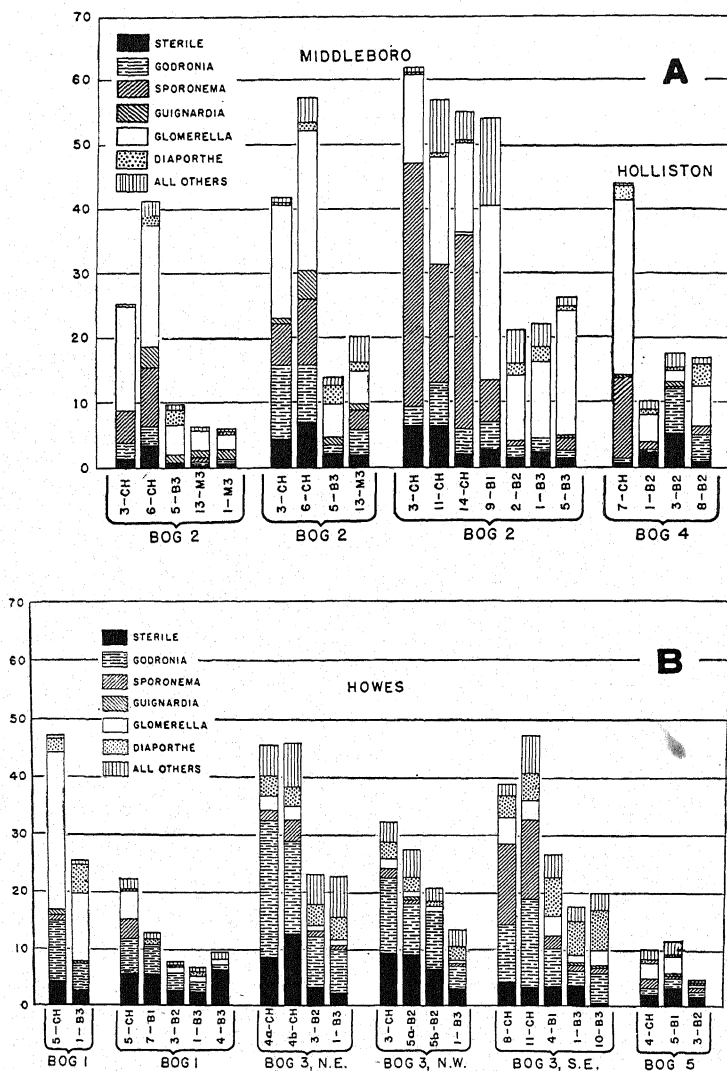


FIG. 1. A. A comparison of the total spoilage of Middleboro and Holliston cranberries from sprayed and check plots, calculated as percentage of yield of the respective plots. The proportion of total spoilage caused by different fungi and by sterile breakdown is shown by sections with special markings, as indicated in the legend. The left group of columns, of the three groups shown for Middleboro bog 2, represents spoilage in the 1932 crop to December 1, 1932; the middle group, spoilage in the 1932 crop to February 1, 1933; and the right group, spoilage in the 1933 crop to November 27, 1933; for Holliston, bog 4, the spoilage is for the 1933 crop to January 1, 1934. Plot numbers and treatment are shown below the columns, Ch—Check, B—Bordeaux, M—Mercurial. Figures following B indicate number of applications during season. B. A comparison of the total spoilage of Howes from sprayed and check plots on three bogs, calculated as percentage of total yield of the respective plots. The two columns of bog 1 at the left represent spoilage in the 1932 crop to February 1, 1933; all others are for the 1933 crop to February 1, 1934.

DISTRIBUTION AND RELATIVE IMPORTANCE OF VARIOUS FUNGI

The total amount of spoilage in berries from nonsprayed plots on any of the bogs studied is no greater than that reported in previous studies (5), except in Early Black from bog 3, where the total spoilage in 1933 amounted to 65 to 95 per cent of the crop. The various bogs differed widely, not only in the total amount of spoilage in berries from them, but also in the relative importance of the several fungi causing it. Early rots caused the greater part of the spoilage of berries from unsprayed plots of a majority of the bogs investigated during the seasons of 1932 and 1933. The most outstanding example is that of the Early Black on bog 3, in which they caused a loss of 60 to 85 per cent of the crop in 1933. There was also an unusually high proportion of loss caused by early rots in berries from nonsprayed plots on bog 2 in 1932 and 1933, and on bog 4 in 1933. Early rots were much less

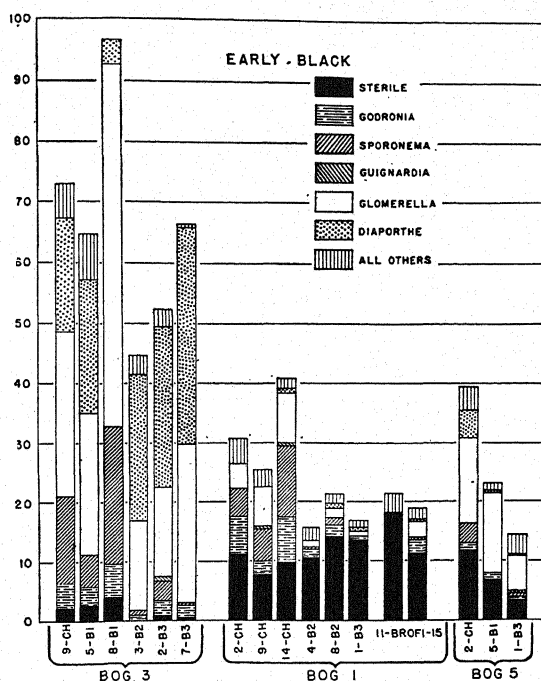


FIG. 2. A comparison of the total spoilage in Early Black cranberries from sprayed and check plots on three bogs, calculated as percentage of yield of the respective plots. The total height of each column represents the percentage of spoilage in the 1933 crop of bog 3 to November 1, 1933; and for the 1933 crop of bogs 1 and 5 to February 1, 1934. Total spoilage and proportion of loss caused by different fungi and by sterile breakdown, indicated as in figure 1. Plot numbers and treatment are shown below the columns, Ch—Check, B—Bodeaux.

important as causes of spoilage in the Howes from N.E. and N.W. plots on bog 3, in 1933, than either *Godronia*¹ or sterile breakdown.

Glomerella, *Sporonema*, and *Diaporthe* were the most important causes of early rots during 1932 and 1933. *Glomerella* was the principal cause of spoilage in berries from nonsprayed plots on bog 2 (Fig. 1, A), and of Howes on bog 1, in 1932 (Fig. 1, B); from plot 9 on bog 2, on bog 4 (Fig. 1, A), and in Early Black on bogs 3 and 5, in 1933 (Fig. 2). Although much less important on other bogs during these 2 years, it was never negligible, but was of minor importance in Howes from bog 3, in 1933.

On some bogs *Sporonema* caused as much or more loss than *Glomerella*. Rot caused by *Sporonema* resulted in a loss of 30 to 38 per cent of the berries of 3 nonsprayed plots on bog 2, in 1933. This is greater than that caused by all other causes combined. It was much less important on this bog in 1932, but, in 1933, was the most important single cause of loss in Howes from nonsprayed S.E. plots on bog 3. Although listed by Shear, Stevens, and Bain (5) under "Important Rot Fungi" and stated (p. 8) to be "A rather common cause of storage rot of cranberries in all cranberry sections" it was not of sufficient importance in their work during a 4-year test to be mentioned as a cause of spoilage (5, Fig. 35; 6, 7, 8); and, as shown on page 35, appeared in less than 5 per cent of the cultures up to November 1 during the 4-year period. Rudolph and Franklin (1) found *Sporonema* to be the least important of 4 fungi as a cause of spoilage in cranberries in 1916 and 1917. They found that most of the spoilage caused by this fungus was in the Early Black. Their results are expressed as percentage of spoiled berries and, although indicating the relative abundance of different fungi, have no absolute value in terms of percentage of spoilage of the storage sample, or in terms of crop loss.

In berries from most of the bogs studied during 1932 and 1933 *Diaporthe* caused less rot than either *Glomerella* or *Sporonema*. The loss caused by it was not more than 2 to 4 per cent, except in the Early Black from several plots on bog 3, in 1933, where it caused a loss of 18 to 35 per cent of the crop (Fig. 2). On these plots, except one, the loss caused by it equalled or exceeded that due to either *Glomerella* or *Sporonema*.

Guignardia was much less important as a cause of fruit rot, on the bogs studied during the 2 years, than any of the 3 fungi named above. It caused a spoilage of more than 1 per cent of the yield in berries from a plot on bog 2 only, in 1932. This fungus has been recognized as of appreciable importance as a cause of early rot in berries from Massachusetts. In the 1927 crop of Howes and McFarlin (5, Fig. 35) it caused the spoilage of approximately 11 per cent of the storage samples.

¹ *Glomerella*, *Sporonema*, *Godronia*, etc., refer to fungus species as published by Shear, Stevens and Bain (5).

It is evident that the importance of any given fungus varies independently of that of any other, and varies not only on different bogs but also on different parts of a particular bog. These variations in the local abundance of specific fungi must be due to local differences in conditions that favor the development of one fungus rather than another. High temperatures during the growing season promote the further development of whatever fungus becomes dominant on any particular bog or plot. The effect of various factors on the development of fungi in competition or association with one another, under field conditions, is at present not known. Among the factors concerned it seems probable that bog management, particularly with respect to the use of water, is very important. For example, bog 3 was reflowed in June, 1933, to which operation the large amount of spoilage in the Early Black on this bog was probably mainly due. Bog 1 was reflowed from June 12 to 14, 1932, which caused a high percentage of spoilage in the berries, as shown in the graph for plot 5 of Howes (Fig. 1, B). The winter flood on this bog was held until May 23, 1933. The total spoilage of berries from plot 5 (Fig. 1, B) in 1933 was less than half that of the previous year, despite much higher temperatures during May and June and the greater number of rainy days during July and August. The reduction in the amount of loss due to *Glomerella* in berries from plot 5, in 1933, as compared with the loss due to this fungus in 1932, is very striking.

CONTROL OF FRUIT ROTS BY SPRAYING

Spraying was beneficial on all bogs on which experiments were conducted in 1932 and 1933, although much variation in the degree of control on different bogs and even on different plots on a given bog was observed. In most instances the amount of rot in berries, at picking time, from plots that had been sprayed 2 or 3 times was reduced to 25 per cent or less of that found in adjacent nonsprayed plots. The benefit of spraying was evident, not only at this time, but continued throughout the storage season. In many instances 2 applications of 4-6-50 Bordeaux mixture² gave apparently as good control of rots as did 3. This, as well as other variations in the apparent degree of control of fungous rots, may be due in part to differences in the abundance of fungi on different plots. One application of Bordeaux usually caused some reduction in the amount of rot but was never so effective as 2, and in some instances apparently produced no effect. Two mercurial sprays, phenyl mercury acetate and ethyl mercury arsenate, used on bog 2 in 1932, were fully as effective as Bordeaux in controlling rots. In one instance only, that of Early Black on bog 3, the control of rots by spraying with Bordeaux was notably poor.

² Equivalent to 4-4-50 of the old formula when stone lime was used.

An analysis of the effects of spraying with reference to the control of specific fungi shows that, in some instances, spraying brought about little or no reduction in the amount of spoilage caused by certain fungi, while in other instances rots were controlled very effectively by the same treatment. *Glomerella* and *Diaporthe* were the fungi that were controlled least. The failure to control these organisms accounts for the notably poor results of spraying the Early Black on bog 3 mentioned just above. Also, spraying had no evident effect on the amount of spoilage due to *Glomerella* in the Middleboro variety from bog 2, in 1933. A similar relation with reference to *Diaporthe* was observed in Howes from S.E. plots on bog 3, in 1933. It is possible that in some instances that first application of spray may have been made somewhat later than it should have been to secure effective control of these fungi. Thus in 1933, it was estimated that 5 to 10 per cent of the buds of Early Black on bog 3 had opened before the first spray was applied. Also, on bog 2, in 1933, many of the flowers were in bloom when the first spray was applied. Studies made since then indicate that infection of flower buds by fungi causing fruit rots occurs at an early stage of bud development. Moreover, bog 3 was reflowed for 32 hours on June 9 and 10 and the first spray was not applied until June 22 so that a great deal of infection had probably taken place before the spray was applied. In all other instances spoilage due to *Glomerella* as well as to other fungi was greatly reduced by spraying, particularly by three applications. *Glomerella* was effectively controlled on bog 2, in 1932.

In both 1932 and 1933, spoilage due to *Godronia* was found very much less in berries from plots sprayed 3 times than from adjacent nonsprayed plots. This is particularly noticeable in a comparison of graphs for plots 3 and 6 with plot 5 on bog 2, in 1932; for plots 4a and 4b with plot 1 N.E., for plot 3 with plot 1 N.W., and for plots 8 and 11 with plots 1 and 10 S.E. of Howes on bog 3, in 1933; for plots 2 and 14 with plot 1 of Early Black, and for plot 5 with plot 4 of Howes on bog 1, in 1933. Two applications of spray, however, had little or no effect on the control of *Godronia*. This indicates that infection by this fungus occurs during the latter part of the summer and that a late spray is perhaps necessary for its control.

Although it was demonstrated by early experiments that spraying usually improves the keeping quality of cranberries in Massachusetts (2, 3), growers have not adopted the practice of spraying. The principal reason for this probably is that the generally good keeping quality of cranberries from most bogs makes it doubtful if there is sufficient benefit to warrant the cost of spraying. This is undoubtedly true for the majority of bogs in Massachusetts, but there are many bogs on which spraying might be done with profit. The amount of improvement obtained in 1932 and 1933 would make a material increase in the net income of the grower by reducing the

amount of rot in the field, in storage before shipment, and particularly in the reduction of adjustments and rejections as a result of spoilage after shipment. On bogs producing cranberries that are below the average in keeping quality, spraying should be adopted as a recognized practice in bog management.

SUMMARY

Early rots caused the greater part of the spoilage of berries from unsprayed plots of a majority of the bogs investigated during the seasons of 1932 and 1933.

Glomerella, Sporonema, and Diaporthe were the most important causes of early rots in 1932 and 1933. Sporonema was much more important as a cause of fruit rot than has previously been reported. Diaporthe caused a loss of 18 to 35 per cent of the crop from several plots on one bog in 1933.

The importance of any given fungus varies independently of that of any other and varies not only on different bogs, but also on different parts of a particular bog.

A single application of 4-6-50 Bordeaux mixture³ was often ineffective and was never so effective as two applications.

Two and three applications of spray were beneficial on all bogs except one on which experiments were conducted in 1932 and 1933, although much variation in the degree of control was observed.

In many instances two applications of Bordeaux gave apparently as good control as three.

Two mercurial sprays, phenyl mercury acetate and ethyl mercury arsenate, used on one bog in 1932, were fully as effective as Bordeaux in controlling rots.

With reference to the control of specific fungi by spraying it was found that Glomerella and Diaporthe were controlled least, and Sporonema was effectively controlled by two or three applications of spray.

The failure to control rots in Early Black on one bog in 1933, by three applications of Bordeaux, is probably due to the fact that the bog was reflooded early in June, prior to the first application of spray.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
EAST WAREHAM, MASS.

³ Equivalent to 4-4-50 with stone lime.

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SPECIFICITY OF ACQUIRED IMMUNITY FROM TOBACCO-RING-SPOT DISEASES¹

W. C. PRICE

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INTRODUCTION

In the last few years a number of workers, including Thung (23), Salaman (19), Oortwijn Botjes (12), Kunkel (10), Ainsworth (1), Caldwell (2), Price (15), and McKinney (11), have presented evidence to show that plant tissues invaded by one strain of a plant virus are immune from infection by other strains of the same virus. The evidence also shows that this immunity is specific in that it does not extend to any of the viruses that are not related to the one used for immunization.

There are 4 virus diseases from which certain species of plants are known to recover and acquire immunity. Three of these, ring spot, green ring spot, and yellow ring spot, have been described previously (26, 24, 14). The fourth, which is designated as ring spot No. 2, was recently isolated by the writer. Since these 4 diseases are alike in many respects, it was suspected that their causal agents might be closely related. It was, therefore, decided to use the immune reaction as an aid in determining the relationship of these viruses and at the same time test the specificity of acquired immunity from the tobacco ring spots by including a number of other viruses which, either from previous work or because of differences in symptoms, host range, and properties, were considered to be entirely distinct from those of the ring-spot group.

SOURCE OF VIRUSES

The viruses used, together with the sources from which they were obtained, and references to descriptions in the literature, are listed in the tabulation on page 666.

The ring-spot virus obtained from Dr. Wingard is designated as ring spot No. 1 in order to avoid confusion with the virus isolated by the writer and designated as ring spot No. 2. Tomato-aucuba-mosaic virus is now recognized as a strain of ordinary tobacco-mosaic virus (10). Southern celery-mosaic virus has recently been shown to be a strain of cucumber-mosaic virus (16). Potato-vein-banding virus was reported by Chester (3) to be serologically identical with cucumber-mosaic virus. Potato-ring-spot virus is believed to be a strain of latent-mosaic virus.

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<i>Virus</i>	<i>Source</i>	<i>Literature citation</i>
Tobacco ring spot No. 1	S. A. Wingard	(26), (14), (17)
Tobacco green ring spot	W. D. Valleau	(24)
Tobacco yellow ring spot	W. D. Valleau	(24)
Tobacco ring spot No. 2	Recently isolated by the writer	
Tobacco mosaic	James Johnson	
Tomato aucuba mosaic	L. O. Kunkel	(10)
Cucumber mosaic	R. H. Porter	(13)
Southern celery mosaic	F. L. Wellman	(5), (25)
Potato vein banding	E. S. Schultz	(8)
Potato ring spot	E. S. Schultz	(9)
Tomato spotted wilt	From a grower in Oregon	(20), (21), (4)
Tobacco etch	W. D. Valleau	(8)
Tobacco severe etch	W. D. Valleau	(8)

DIFFERENTIATION OF THE RING-SPOT VIRUSES

The symptoms caused by most of the viruses used in the present study are so well known that a description of them need not be given here. A description will, however, be given of the symptoms of tobacco ring spot No. 2, since there is no previous reference to this disease in the literature. In addition, it is believed desirable to point out some of the similarities and differences in symptomatology between tobacco ring spot No. 1, green ring spot, and yellow ring spot.

Differentiation of Ring Spot No. 1, Green Ring Spot, and Yellow Ring Spot. The 3 diseases may be distinguished by the symptoms they produce in 2 host plants, tobacco (*Nicotiana tabacum* L. var. Turkish) and cowpea (*Vigna sinensis* Endl.). Yellow ring spot is distinguished from both ring spot No. 1 and green ring spot by the fact that it produces bright yellow, as well as necrotic, systemic lesions in tobacco, whereas the other 2 diseases produce only necrotic or pale yellow lesions. It also is characterized by the fact that old leaves of plants that have recovered from the disease are yellow in color, particularly on their margins, while leaves of plants that have recovered from either ring spot No. 1 or green ring spot are dark green. Ring spot No. 1 differs from green ring spot in producing more severe symptoms in tobacco, considerably more necrosis, and somewhat greater stunting. Plants recover from ring spot No. 1 earlier than from either green or yellow ring spot, suggesting that the virus of ring spot No. 1 moves more rapidly in tobacco than either of the other 2 viruses.

The primary lesions produced by ring spot No. 1 in cowpeas are usually solid necrotic spots and only infrequently fail to become completely necrotic, while those caused by green or yellow ring spot are, as a rule, necrotic only around their peripheries. The virus of ring spot No. 1 moves more rapidly

and causes more injury in the cowpea than the virus of either green or yellow ring spot. In a set of cowpea plants inoculated with one or another of the 3 viruses, all the plants infected with ring spot No. 1 were dead on the 9th day after inoculation, while those infected with green and yellow ring spot were alive on the 13th day.

Tobacco Ring Spot No. 2. In the summer of 1934, and on several subsequent occasions, there appeared in a greenhouse at Princeton a ring-spot disease in 1 or 2 plants in an otherwise healthy set of Turkish tobacco. The virus of this disease is readily transmitted by the leaf-rubbing method of inoculation. In tobacco it produces primary lesions consisting of zonate necrotic spots similar to but distinct from those produced by tobacco ring spot No. 1 (Fig. 2). The disease likewise produces systemic lesions of the same type, and plants affected systemically by it eventually recover in much the same manner that tobacco plants recover from tobacco ring spot No. 1 (14). On the other hand, the disease may frequently become localized, in which case the virus fails to reach the tip portions of the plant. It is usually possible to induce systemic symptoms and subsequent recovery by cutting back the plants or by fertilizing them heavily. Tobacco plants that have recovered from ring spot No. 2 are immune from a second attack of the disease.

Ring spot No. 2 was transmitted to *Nicotiana glutinosa* L., *N. langsdorffii* Weinm., *N. sylvestris* Spegaz. and Comes, tomato (*Lycopersicon esculentum* Mill.), bean (*Phaseolus vulgaris* L.), and cowpea, and was characterized by the production of primary and systemic necrotic lesions in these hosts. The subsequent behavior of the disease was not extensively studied.

Evidence was obtained that virus of ring spot No. 2 may be transmitted through seed of Turkish tobacco to a small percentage of progeny from diseased plants. A careful observation of approximately 5,000 seedlings grown from seed of plants that had recovered from the disease revealed no symptoms in any of them. The seedlings were then divided into lot of about 125 each, juice being expressed from each lot and tested separately for presence of virus by inoculation of 6 Turkish tobacco plants. Infection was obtained from only one of the 40 lots of plants, and in this instance symptoms typical for the virus of ring spot No. 2 were obtained. While the evidence for seed transmission is not conclusive, it is at least suggestive that the virus may occasionally be transmitted through seed of Turkish tobacco.

Tobacco ring spot No. 2 is similar in some respects to the necrotic virus disease described by Smith and Bald (22). A sample of the virus was sent to Dr. K. M. Smith who reported that he did not believe it to be identical with his necrotic virus nor with any of the other viruses in his collection.

TESTS FOR ACQUIRED IMMUNITY IN TURKISH TOBACCO
AND *NICOTIANA SYLVESTRIS*

All the experiments to be reported on specificity of acquired immunity from tobacco-ring-spot diseases were repeated on one or more occasions. The results obtained from the repetitions were similar to, and served to confirm, the results obtained in the first experiments.

Ring-spot Diseases. Experiments were conducted for the purpose of determining whether or not plants that had recovered from any one of the 4 ring-spot diseases would be immune from either of the other 3. In one set of experiments, Turkish tobacco was used as a host plant and in another set *Nicotiana sylvestris*. Since similar results were obtained with these 2 hosts, they will be considered together.

For the experiments with Turkish tobacco, tip cuttings were taken from healthy control plants and from plants that had recovered from ring spot No. 1, from green ring spot, from yellow ring spot, and from ring spot No. 2, respectively. They were rooted in moist peat moss and sand and then potted in 4-inch pots. When they had attained a suitable size, 5 plants of each of the 5 groups were heavily inoculated with one of the ring-spot viruses by means of the rubbing method.

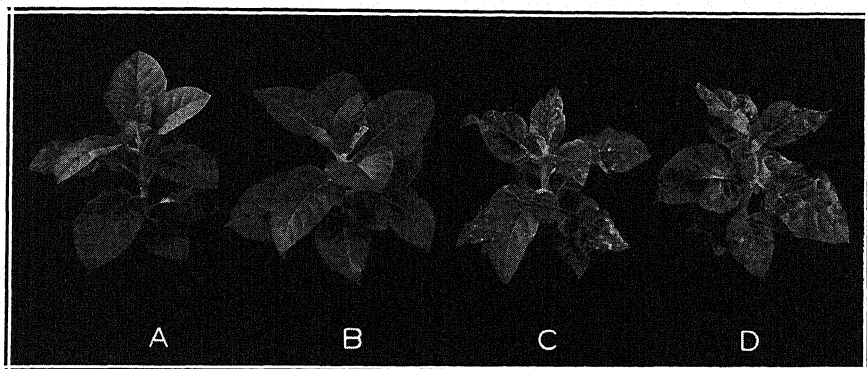
For the experiments with *Nicotiana sylvestris*, cuttings grown from recovered and from healthy plants were divided into sets each of which consisted of 5 plants that had recovered from ring spot No. 1, 5 that had recovered from yellow ring spot, and 5 controls. One set of plants was inoculated with ring spot No. 1, another with yellow ring spot, a third with green ring spot, and a fourth with ring spot No. 2.

TABLE 1.—Summary of tests for immunity in plants of Turkish tobacco and *Nicotiana sylvestris*

Virus used for inoculation	Tests with Turkish tobacco					Tests with <i>N. sylvestris</i>		
	Plants recovered from				Controls	Plants recovered from		Controls
	Ring spot No. 1	Green ring spot	Yellow ring spot	Ring spot No. 2		Ring spot No. 1	Yellow ring spot	
Ring spot No. 1.....	++ ^a	++	++	0	0	++	++	0
Green ring spot.....	++	++	++	0	0	++	++	0
Yellow ring spot...	+	+	++	0	0	+	++	0
Ring spot No. 2.....	0	0	0	++	0	0	0	0

^a 0 = no protection, + = partial protection, ++ = complete protection.

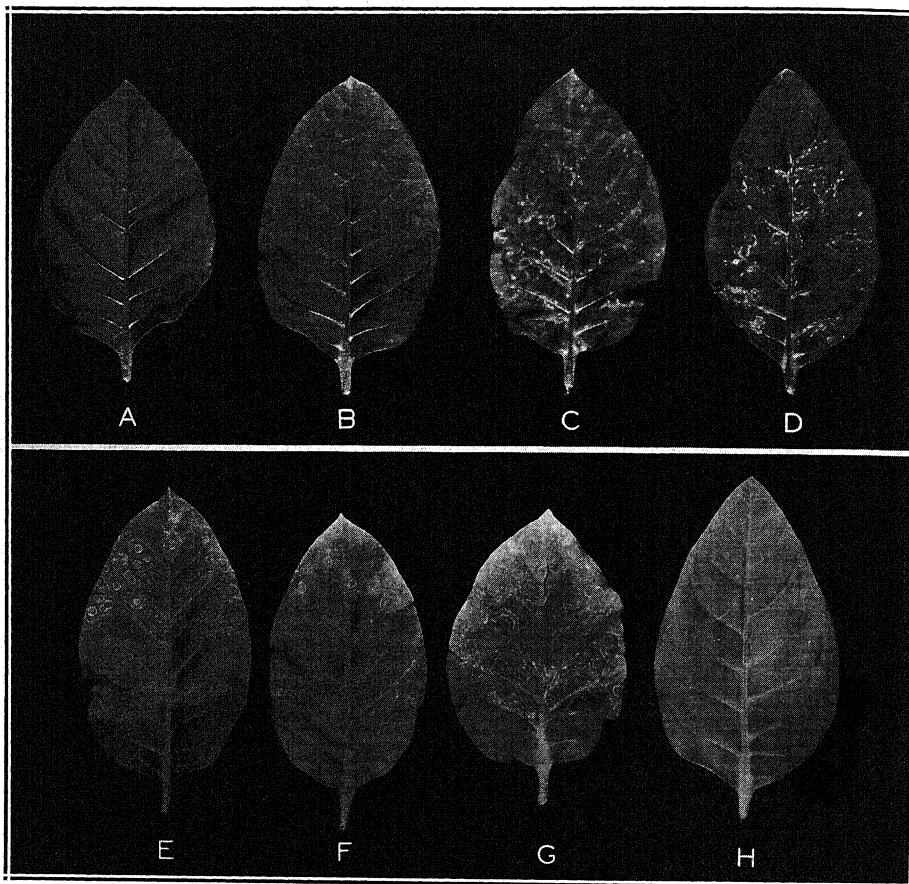
Table 1 summarizes the results obtained. Three of the viruses, ring spot No. 1, green ring spot, and yellow ring spot, gave protection against one another, but did not protect against ring spot No. 2. Plants that had recovered from the latter were likewise susceptible to the other ring-spot diseases, but were solidly immune from ring spot No. 2. The findings are illustrated in figures 1 and 2. Figure 1 shows the results obtained when ring spot No. 1 was used as inoculum, figure 2 (A to D) the results when green ring spot was used, and figure 2 (E to H) the results when ring spot No. 2 was used.



Photographed by J. A. Carlile

FIG. 1. Turkish tobacco plants grown from cuttings and inoculated with virus of ring spot No. 1. A. A plant that had recovered from ring spot No. 1. B. A plant that had recovered from green ring spot. C. A plant that had recovered from ring spot No. 2. D. A previously healthy plant. The photograph was taken 10 days after inoculation.

It will be noticed from table 1 that ring spot No. 1 gave complete protection against green ring spot and that green ring spot gave complete protection against ring spot No. 1, but that neither ring spot No. 1 nor green ring spot completely protected plants from infection with yellow-ring-spot virus. When plants that had recovered from ring spot No. 1 were inoculated with yellow-ring-spot virus, they showed no symptoms during the first 9 days after inoculation, but on the 10th day they exhibited a number of small, yellow, primary lesions. The lesions increased somewhat in size, and in a few instances developed partially necrotic edges. Systemic lesions were observed 40 days after inoculation and consisted of small yellow spots with zonate rings. As the plants became older, more and more yellow lesions appeared in the young leaves, so that eventually they were almost entirely covered by small yellow areas. At a still later stage the doubly infected plants recovered from the yellow-ring-spot disease and produced leaves similar in appearance to those produced by plants that had recovered from



Photographed by J. A. Carlile

FIG. 2. Turkish tobacco leaves inoculated with virus of green ring spot (A to D) and with virus of ring spot No. 2 (E to H). A, E. From a plant recovered from ring spot No. 1. B, F. From a plant recovered from green ring spot. C, G. From a previously healthy plant. D, H. From a plant recovered from ring spot No. 2. The picture was taken 10 days after inoculation.

yellow ring spot alone. Figure 3 illustrates the lesions obtained in *Nicotiana sylvestris* one month after inoculation.

When plants that had recovered from green ring spot were inoculated with yellow-ring-spot virus, they showed no symptoms for a month after inoculation. A few discrete yellow lesions then appeared in some of the inoculated leaves. Similar lesions developed later in the young noninoculated leaves. In other instances, no symptoms were produced within the period of more than 2 months that the plants were kept under observation.

Ring Spot and Other Diseases. Experiments were undertaken to deter-



Photographed by J. A. Carlile

FIG. 3. *Nicotiana sylvestris* plants grown from cuttings. Left, a plant recovered from ring spot No. 1 and inoculated with virus of yellow ring spot. Center, a previously healthy plant inoculated with virus of yellow ring spot. Right, a healthy control. Note the small lesions in the leaves of the plant on the left and compare with the large lesions present in the center plant. The picture was taken one month after inoculation.

mine, firstly, whether or not the ring-spot viruses would protect tobacco plants from infection with 9 other viruses, and, secondly, whether or not these other viruses would afford protection against the viruses belonging to the ring-spot group. The experiments were similar to those reported in the previous section.

The viruses used for inoculation of plants that had recovered from one or another of the ring-spot diseases were tobacco mosaic, aucuba mosaic, cucumber mosaic, celery mosaic, potato vein banding, potato ring spot, tomato spotted wilt, etch, and severe etch. In the reciprocal tests, the virus of spotted wilt was not included because of the difficulty of obtaining a thorough systemic infection with it in tobacco. Also, celery-mosaic and aucuba-mosaic viruses were not included because of their relationship to cucumber-mosaic and tobacco-mosaic viruses, respectively.

The results of the experiments may be summarized briefly as follows: The ring-spot viruses gave no protection against any of the other viruses tested, nor did any of these other viruses protect plants from infection with any of the ring-spot viruses. The symptoms of tobacco mosaic in plants that had recovered from ring spot No. 1, from green ring spot, or from yellow ring spot were milder than those produced in the control plants. A similar modification of tobacco-mosaic symptoms has been reported previously (14). The symptoms of etch in plants that had recovered from ring spot No. 1 were also milder than those in the control plants. In most instances, the lesions produced by the ring-spot viruses in plants infected with the other viruses tested were somewhat less numerous than those produced in the controls. Systemic symptoms of the ring-spot viruses were slightly delayed in plants

infected with severe etch as compared with the controls. In other respects, the symptoms produced by the ring-spot viruses in plants infected with the other viruses tested, and those produced by these other viruses in plants that had recovered from one or another of the ring-spot diseases, were no different from the symptoms produced in the control plants.

The conclusions to be derived from the results of these experiments may be summarized briefly as follows: Firstly, the fact that ring spot No. 1 and green ring spot protect completely against each other indicates that they are closely related strains belonging in the same virus group. Secondly, the fact that yellow ring spot protects completely against ring spot No. 1 and against green ring spot, together with the fact that both ring spot No. 1 and green ring spot afford a partial protection from yellow ring spot, suggests that the viruses of the 3 diseases are related. This conclusion is in accord with that reported by Chester (3), who found a close serological relationship between the virus of ring spot No. 1 and yellow ring spot. Thirdly, the virus of tobacco ring spot No. 2 must be considered as entirely distinct from all the other viruses tested. Finally, the failure of the ring-spot viruses to induce immunity against any one of 9 viruses outside the ring-spot group brings evidence for the specificity of the immune reaction.

Tests with Plants Recovered from both Ring Spot No. 1 and Ring Spot No. 2. An experiment was performed for the purpose of determining whether or not plants that had recovered from ring spot No. 1 and also from ring spot No. 2 would become immune from both diseases. Cuttings were grown from 5 plants that had recovered from ring spot No. 1, from 5 plants that had recovered from ring spot No. 2, and from 10 healthy plants. The 5 plants that had recovered from ring spot No. 1 and 5 of the control plants were inoculated with virus of ring spot No. 2. Similarly, the 5 plants that had recovered from ring spot No. 2 and the remaining 5 control plants were inoculated with virus of ring spot No. 1. The plants in all 4 sets became infected, developed symptoms, and in due time recovered. Five of the plants that had recovered from both ring spot No. 1 and ring spot No. 2 and the 5 plants that had recovered only from ring spot No. 1 were inoculated with virus of ring spot No. 2. Symptoms developed in the plants that had recovered from ring spot No. 1 alone, but not in the plants that had recovered from both diseases. The remaining 5 plants that had recovered from both ring spot No. 1 and ring spot No. 2 and the 5 plants that had recovered from ring spot No. 2 alone were inoculated with virus of ring spot No. 1. No symptoms developed in the plants that had recovered from both diseases, but the plants that had recovered only from ring spot No. 2 came down with a severe infection of ring spot No. 1. The results show that, while tobacco plants recovered from only one of the 2 ring-spot diseases are susceptible to the other, plants that have recovered from both diseases are immune from both.

DISCUSSION

The results reported here show that acquired immunity from tobacco ring spot is highly specific. Plants that have recovered from ring spot No. 1 are immune from this disease and from green ring spot, and they are highly resistant to yellow ring spot. They are not, on the other hand, immune from tobacco ring spot No. 2, tobacco mosaic, aucuba mosaic, cucumber mosaic, celery mosaic, potato vein banding, potato ring spot, tomato spotted wilt, etch, or severe etch. The results have likewise shown that the immunity acquired by plants that have recovered from ring spot No. 2 extends only to this disease and not to any of the others studied.

The failure of ring spot No. 1 to protect completely against yellow ring spot is of interest because it indicates a possibility that yellow ring-spot virus has associated with it a property peculiar to itself in addition to properties also associated with ring spot No. 1. The virus of the latter disease has the power to induce immunity from ring spot No. 1, but not a complete immunity from yellow ring spot, while yellow-ring-spot virus induces immunity not only from yellow ring spot but also from ring spot No. 1.

If the degree of protection afforded by the immune reaction may be taken as an indication of the closeness of relationship of plant viruses, then the evidence suggests that yellow ring spot is more closely related to green ring spot than it is to ring spot No. 1. Plants that have recovered from green ring spot appear to be less susceptible to yellow ring spot than are plants that have recovered from ring spot No. 1. To a certain extent this conclusion is supported by other lines of evidence. While the necrotic lesions produced in cowpea by green- and yellow-ring-spot viruses are practically identical, they differ from those induced in the same host by virus of ring spot No. 1. It has been shown (24) that green- and yellow-ring-spot viruses are transmitted through seed of tobacco to as much as 15 per cent of progeny from recovered plants. On the other hand, no evidence has been obtained that the virus of ring spot No. 1 is transmitted through seed of Turkish tobacco (18, 14, 7), although it has been shown to be transmitted through seed of petunia (6).

It is of considerable interest that there are at least 2 distinct virus diseases from which tobacco plants regularly recover, and that in the case of both of these the plants are immune from a second attack. It may be of some significance that these diseases are much alike in their symptomatology, in their host ranges, and in other ways. However, if there is a connection between recovery from a disease and some particular characteristic of the disease, it remains to be discovered. On the other hand, it is apparent from a study of tobacco ring spot No. 1 and tobacco ring spot No. 2 that either of these diseases interferes little or not at all with the other. A plant that has recovered from one is highly susceptible to the other and, once infected,

develops severe symptoms, which are followed sooner or later by recovery. Plants that have recovered from both diseases are immune from both.

SUMMARY

A virus disease of tobacco, designated as ring spot No. 2, was shown by means of the immune reaction to be entirely distinct from ordinary tobacco ring spot, designated as ring spot No. 1, and from green and yellow ring spot. Tobacco plants infected with ring spot No. 2 recover and develop a solid immunity from the disease. They do not become immune from tobacco ring spot No. 1 or from any of the 11 other virus diseases tested.

Ring spot No. 1 and green ring spot give complete protection against each other, but neither protects completely against yellow ring spot. On the other hand, yellow ring spot protects against ring spot No. 1 and green ring spot. These facts indicate that ring spot No. 1, green-ring-spot, and yellow-ring-spot viruses are closely related.

Ring spot No. 1, green ring spot, and yellow ring spot in tobacco gave no protection against tobacco-mosaic, aucuba-mosaic, cucumber-mosaic, celery-mosaic, potato-vein-banding, potato-ring-spot, spotted-wilt, etch, or severe-etch viruses. The results bring evidence for the specificity of acquired immunity from tobacco-ring-spot diseases.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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PYTHIUM GRAMINICOLUM AND P. ARRHENOMANES

CHARLES DRECHSLER

(Accepted for publication August 7, 1935)

In a recent paper Carpenter (3), after a consideration of literature on species of *Pythium* responsible for root injury, more particularly to graminaceous hosts, set forth the conclusion that the parasite associated in Hawaii with growth failure of sugar cane (*Saccharum officinarum* L.)—a parasite he had first referred to as *P. butleri* Subr., and later as *P. aphanidermatum* (Eds.) Fitz.—was identical with both *P. graminicolum* Subr. and *P. arrhenomanes* Drechsl. Subramaniam's statement (12) that the fungus isolated in India from diseased roots of wheat (*Triticum aestivum* L.) agreed with the description and drawings of the *Pythium* destructive to sugar cane in Hawaii (2), together with Matthews' corroborative citation of the "*P. aphanidermatum* of Carpenter" as a synonym of *P. graminicolum* (9), was held to establish identity with the one species; while the inclusion recently by Rands and Dopp (11), of an actual culture isolated in Hawaii from diseased sugarcane roots among the strains of *P. arrhenomanes* studied by them, was interpreted quite justifiably as establishing identity with the other. Not illogically, this dual identification was held to imply identity of the two species themselves; wherefore, it presumably remained only to determine which of the two had been described the earlier to decide upon the name by which the fungus might be correctly designated. As priority appeared to favor Subramaniam's description over my own, *P. graminicolum* was adopted as the valid binomial; *P. arrhenomanes*, at the same time, being reduced to synonymy.

If the dispositions thus made were to be found in satisfactory agreement with the facts of morphology, the resulting simplification in taxonomy would be more welcome even than the abasement of a species name somewhat indecent in its spiritual connotation. It can not be doubted that the identification of the Hawaiian fungus used by Rands and Dopp as *Pythium arrhenomanes* was altogether correct. The detailed study by these authors, of numerous strains of the species both in comparison with each other and with many strains referable to a dozen other species of *Pythium*, could hardly have failed to supply more than adequate information and experience. On the other hand, Subramaniam's judgment as to the scope of his species relative to the fungus set forth as a parasite on sugar cane in Hawaii, was apparently based solely on information conveyed in Carpenter's early account. With respect to what would seem to constitute the most decisive morphological feature concerned here, the origin and mycelial connections of the antheridia, this account unfortunately is somewhat less clear than

might be desired. In the illustrations accompanying it, the parts probably meant to represent antheridia show no indication of being set off by basal septa; and many of the elements thus meagerly differentiated are shown juxtaposed to the oogonium in a manner leaving doubt as to whether structural continuity with, or disappearance beneath, the female organ was intended to be depicted. A degree of ambiguity is thereby introduced that would seem to make reference of the description to any particular one of several likely species about equally uncertain. Comparison of Carpenter's figures with those illustrating the original account of *P. graminicolum* reveals so little correspondence in origin of antheridial branches that Subramaniam's ready acceptance of specific identity appears somewhat surprising. Nor is a closer agreement evident when comparison with regard to antheridial connections is extended to Matthews' figures of presumably the same fungus.

Since the culture on which the description of *Pythium graminicolum* was based has long been lost, it is fortunate that the description in itself leaves little uncertainty as to the proper application of the species. The close mycelial connection between oogonium and antheridia, evident in Subramaniam's figure, is paralleled in many cultures that R. D. Rands, beginning in 1927, isolated from diseased roots of sugar cane, together with numerous strains of various other forms, among which, as was intimated in my earlier account (4), *P. arrhenomanes* was abundantly represented. These cultures were, from the beginning, recognized as belonging to a species so distinct from *P. arrhenomanes* that confusion between the two was not expected. The species in question had become known to me some years earlier through a single culture received from B. A. Bourne, who had isolated it in Puerto Rico from a diseased root of sugar cane in connection with investigations reported by him (1) in 1924.

The relationship of parts in the sexual apparatus to the Puerto Rican fungus is illustrated in figure 1. This figure, it may be explained, was drawn in 1925; so that its preparation was influenced neither by Subramaniam's paper, which appeared 3 years later; nor by Matthews' book, issued 6 years later; nor, least of all, by considerations growing out of the issue raised in Carpenter's recent contribution. A parallelism in antheridial connections, with the sexual apparatus of *Pythium graminicolum* as figured by both Subramaniam and Matthews, is, I believe, so readily apparent as to require no further exposition. In appropriately irrigated material the fungus gives rise to lobulate zoosporangia and zoospores likewise corresponding well to the homologous structures of the wheat parasite. It was therefore assigned, together with the morphologically similar cultures obtained from sugar-cane roots in the southern United States, to *P. graminicolum* as soon as the description of that species made its appearance.

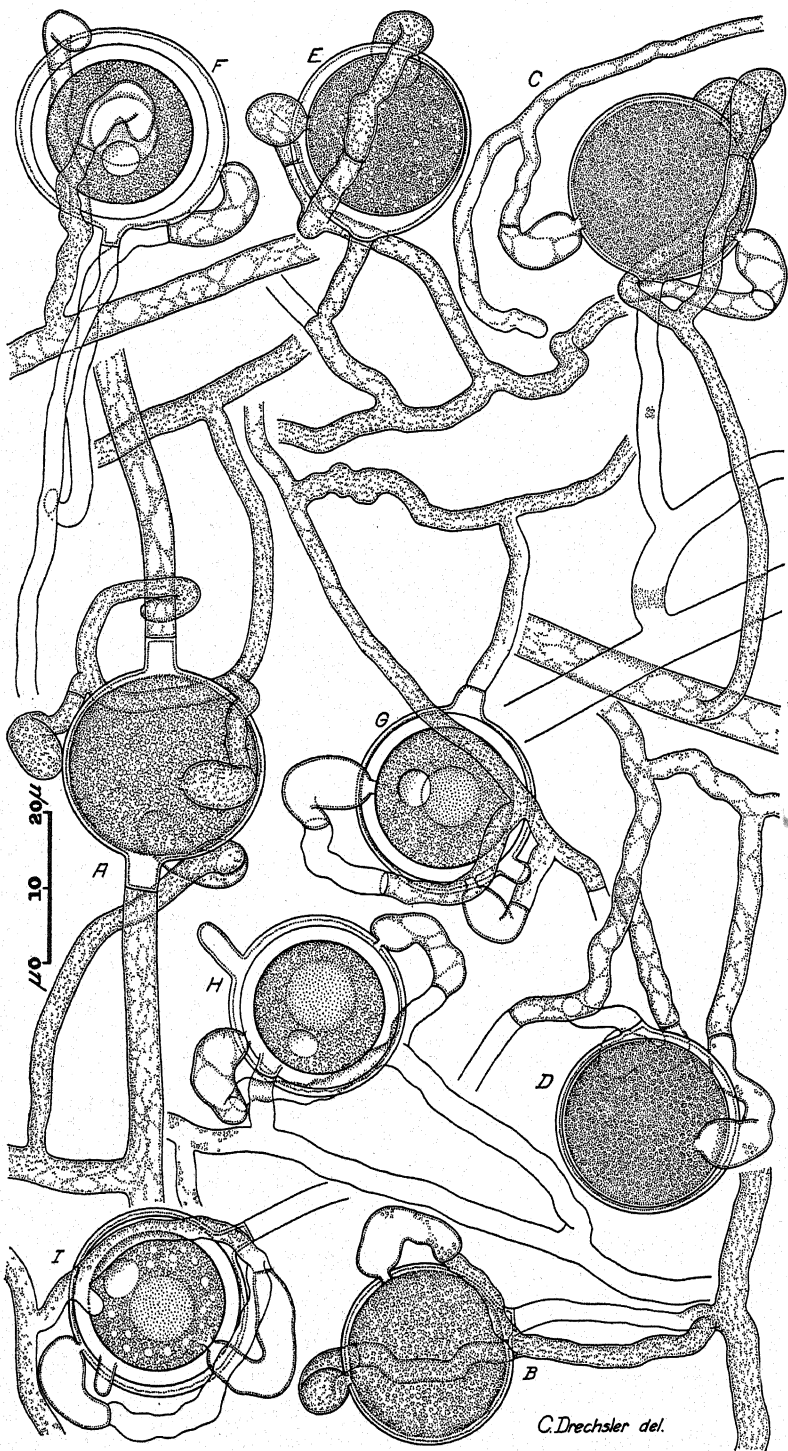


FIG. 1. Sexual apparatus of a Puerto Rican strain of *Pythium graminicolum* developed in a maize-meal agar plate culture, and drawn with the aid of the camera lucida. $\times 1000$.



FIG. 2. Sexual apparatus of the type culture of *Pythium arrhenomanes* on maize-meal agar; drawn with the aid of the camera lucida. $\times 1000$.

A conspicuously different relationship of parts is evident in figure 2, representing sexual apparatus of the type culture of *Pythium arrhenomanes* isolated from diseased roots of maize (*Zea mays* L.) by Helen Johann and employed in investigations reported by Johann, Holbert, and Dickson (7, 8). The mycelial connection between oogonium and antheridium here is much more distant than in *P. graminicolum*; usually, indeed, being too distant to be traced in the tangle of rather irregularly disposed filaments that make up the somewhat characteristic growth in agar plate cultures. In especially favorable material the continuity of hyphal elements between the male and the female organs can occasionally be seen, and, as in homothallic forms generally, such continuity is necessarily always present when a single mycelium occupies a tract of substratum. Rands and Dopp have well pointed out that the type culture produces antheridia more abundantly than most strains of the species. Yet the similarity in origin and arrangement of the male organs is recognizable without difficulty in strains producing these structures in more moderate quantity; as, for example, in the strain represented in figure 3, which was isolated from diseased maize rootlets submitted by W. D. Valleau as being typical of rootrot described from Kentucky by Valleau, Karraker, and Johnson (13). Figures 2 and 3 were drawn in 1927, and may, therefore, also be assumed to have been prepared in the absence of any possible prejudice bearing on the taxonomic issue under discussion.

Associated with the differences in arrangement of sexual apparatus are other differences, which, if less easily expressed in words or in drawings, are often experienced vividly enough when *Pythium graminicolum* and *P. arrhenomanes* are grown side by side on artificial substrata. On agar media containing maize-meal decoction with some of the finer maize-meal sediment, *P. graminicolum* develops a mycelium that in due course usually gives rise to oogonia and antheridia. For the most part each oogonium develops a normal oospore, which at maturity consists of a fairly thick wall, a parietal layer of granular protoplasm, a subspherical or ellipsoidal refringent body imbedded in the parietal layer, and a central vacuole-like reserve globule (Fig. 1, F-I). The oogonial wall, the membrane of each antheridium, and the wall of each supporting hyphal element are so substantial that, after maturity, they, for the most part, long retain their respective original shapes without much alteration from collapse, and long remain very clearly visible to microscopic inspection. Asexual reproduction likewise is easily induced. When pieces of an agar plate culture with vigorous vegetative mycelium are appropriately irrigated, lobulate sporangial complexes are developed that freely give rise to zoospores. Although some strains are more refractory than others, the species, on the whole, readily reveals both of its reproductive stages, which, because of the continuing visibility of the sexual apparatus, are easy to study and to delineate.

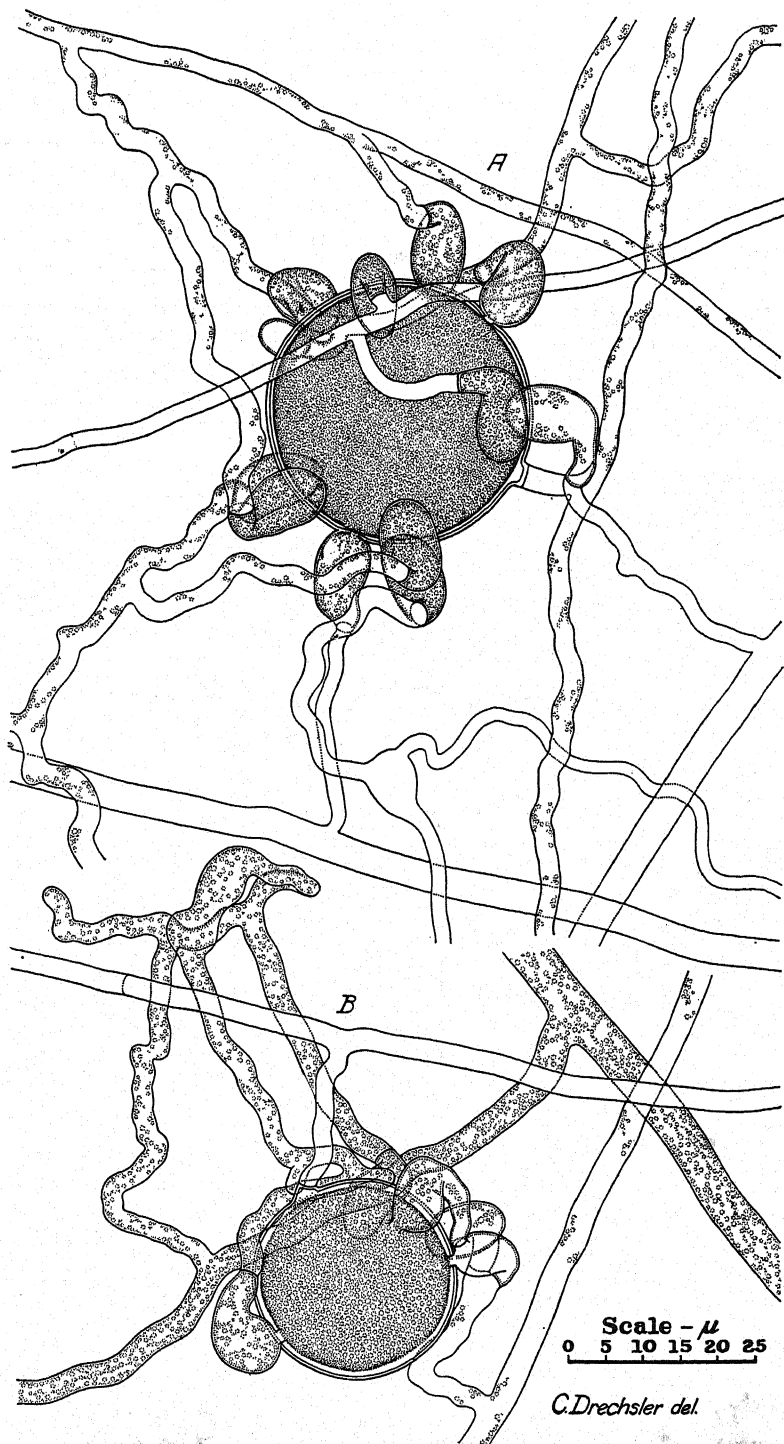


FIG. 3. Sexual apparatus of a Kentucky strain of *Pythium arrhenomanes* developed in a maize-meal agar plate culture, and drawn with the aid of the camera lucida. $\times 1000$.

Pythium arrhenomanes, on the other hand, offers many exasperating difficulties. On ordinary maize-meal agar vegetative growth is profuse, and with many strains sex organs as well as massive lobulate sporangial complexes are soon produced in quantity. But the oogonia, together with the antheridia supplying them, almost without exception undergo degeneration, their contents either breaking down or migrating into hyphal outgrowths. Through the use of special media, like those devised by Johann (6) and by Rands and Dopp (10), the production of sexual apparatus can be stimulated, and its degeneration reduced; some strains, as, for example, the one represented in the type culture, then yielding oospores of normal structure in more than half of the numerous oogonia. Yet, even after normal development has been obtained, the frequently haphazard and rather intricate disposition of the rangy antheridial filaments is not always readily made out. Moreover, after fertilization has been completed, the antheridia and the branches supporting them become increasingly difficult to see; with the result that on maturity of the oospore they have become for the most part nearly indiscernible. The hypha bearing the oogonium similarly becomes less clearly visible, and even the oogonial wall will often lose much of its optical distinctness.

Though massive lobulate complexes are often formed abundantly when *Pythium arrhenomanes* is cultivated on maize-meal agar, these structures, on being irrigated in a manner successful for zoospore production in many congeneric forms, exhaust themselves in a promiscuous proliferation of aerial or submerged filamentous outgrowths, without giving any sign of normal sporangial development. Zoospore formation, it is true, has been observed a few times on irrigating pieces of Lima bean decoction agar occupied by vigorously growing mycelium; the output in all such instances being, however, very meager in comparison with that ensuing when freshly invaded maize roots are placed in water. The strong tendency toward degeneration of reproductive bodies, noticeable on most artificial media in common use, combined with the intricacy of the sexual apparatus and the early evanescence especially of the male parts, has undoubtedly been responsible in no small degree for the unimpressive descriptive treatment that long permitted the species to remain in obscurity.

A closer approach to the morphology and cultural traits of *Pythium arrhenomanes* than is seen in *P. graminicolum*, or, for that matter, in any other form known to me, is evident in a species I have described elsewhere (5) as *P. myriotylum*. The extraordinary haustorial development associated with the frequently aerial parasitism characteristic of this species, and the less pronounced inflation of its sporangial complexes, which, after developing on artificial media, rather readily give rise to zoospores, should suffice, nevertheless, to distinguish it from *P. arrhenomanes*. *P. myriotylum* has

not hitherto been recognized in any collections of ultures derived from diseased roots of graminaceous plants; and in a soil at all compact, would seem incapable of operating at a sufficient depth to attack plant parts far under ground. Its remarkable parallelism in parasitic habit and host range to *P. butleri* may be expected to make for its orientation with reference to that species of much different morphology, rather than for orientation with reference to the fundamentally more similar and apparently more closely related graminicolous form.

SUMMARY

Though *Pythium graminicolum* and *P. arrhenomanes* are both parasitic on sugar cane, being associated abundantly with root rots of this host in the southern United States, they represent separate species rather than a single species, as has recently been averred. A close mycelial connection between oogonium and antheridium, very frequent in *P. graminicolum*, is rare in *P. arrhenomanes*; and in parallel cultures the sturdy, more substantial membranous parts of the sexual apparatus of the former species remain clearly discernible long after the evanescent antheridial envelopes and supporting branches of the latter have become nearly or wholly invisible.

BUREAU OF PLANT INDUSTRY,

U. S. HORTICULTURAL FIELD STATION,

BELTSVILLE, MARYLAND.

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THE OCCURRENCE OF GIBBERELLA FUJIKUROI VAR. SUBGLUTINANS IN THE UNITED STATES¹

ARNOLD J. ULLSTRUP

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INTRODUCTION

Specimens of perithecia that superficially appeared like those of *Gibberella saubinetii* (Mont.) Sacc. were found on old corn stalks in New Jersey and Ohio. Microscopic examination of the fruiting bodies indicated that they were not those of *G. saubinetii*, since the asci were long and relatively narrow, and bore straight, 1-septate ascospores. A few of the asci contained 4 or 6 ascospores instead of the usual 8. These observations suggested that the fungus might be *Gibberella fujikuroi* (Saw.) Wr.² On culturing a number of single ascospores, however, it was found that microconidia were never produced in chains as in *Fusarium moniliforme* Sheld., the imperfect stage of *G. fujikuroi*. Further observations strongly indicated that the fungus was *G. fujikuroi* (Saw.) Wr. var. *subglutinans* Edwards.

Since *Gibberella fujikori* var. *subglutinans* Wr. and Rg. and its conidial stage, *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. and Rg. have never been reported in this country, and since this variety and its species, *G. fujikuroi*, are very similar morphologically, a comparison of the two together with their respective imperfect forms seemed advisable in order to confirm the identity of the fungus in question.³

G. fujikuroi var. *subglutinans* was first reported by Edwards (1) in New South Wales, Australia, in 1933. He found the perithecia on old corn stalks and demonstrated the pathogenicity of the conidial stage on the seedlings, stalks, and ears of corn, *Zea mays* L. The imperfect, or conidial stage, *Fusarium moniliforme* var. *subglutinans*, has been reported on a number of hosts including banana, *Musa sapientum* L. (6), pineapple, *Ananas sativus* Schult. (7), manila hemp, *Musa textilis* Nee (6), sugar cane, *Saccharum officinarum* L. (3), and wheat, *Triticum sativum* Lam. (2). Recently the fungus has been reported from New Zealand as the cause of pink cob-rot of corn (4).

¹ Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

² In a recent treatise on the genus *Fusarium* (9), Wollenweber and Reinking have used the binomial *G. fujikuroi* (Saw.) Wr. in preference to its synonym *G. moniliformis* (Sh.) Winel.

³ The writer is indebted to C. D. Sherbakoff for substantiating the identity of this fungus.

The ascigerous stage of the fungus is closely related morphologically to *Gibberella fujikuroi*, but differs from the latter in that 8-spore asci are more common than the 4- to 6-spore asci, and the 2- to 3-septate ascospores are fewer in number. In addition, the ascospores are more blunt and are short-elliptical in shape. The conidial stage, *Fusarium moniliforme* var. *subglutinans*, is primarily differentiated from the type form, *F. moniliforme* Sheld., of the section *Liseola* in the genus *Fusarium* Link, in that the microconidia are not borne in chains.

EXPERIMENTAL

It is well known that the *Fusaria*, as a group, are extremely variable, and that this variation may be expressed physiologically, morphologically, and in cultural characters. In spite of this variability, it was believed that certain differences, especially of the more stable characters, might be brought out between the two forms.

Morphology. The morphological characteristics of the ascigerous stage of *Gibberella fujikuroi* var. *subglutinans* were studied as the fungus occurred on dead corn stalks. The conidial stage was studied while growing on Lima bean agar in pure culture. All cultures were of single ascospore origin.

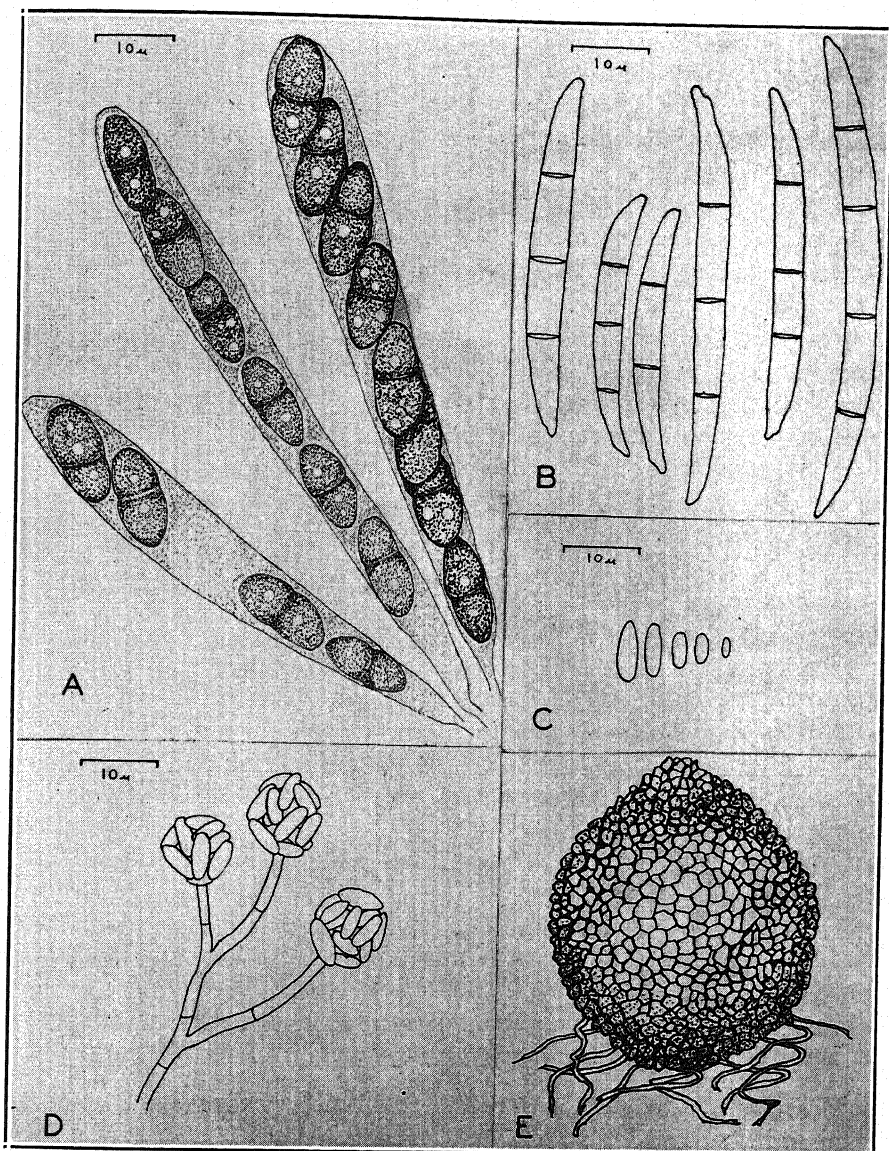
The following are measurements of conidia from a 15-day-old colony grown on Lima bean agar at room temperature (22–24° C.). The average spore size in each of the following groups is based on the measurements of 100 conidia selected at random:

0—septate	10.1 × 2.9 μ	(7.4–14.9 × 2.4–3.3 μ)
1—septate	20.0 × 3.3 μ	(14.9–26.5 × 2.4–3.7 μ)
2—septate	25.1 × 3.7 μ	(21.5–33.2 × 3.3–4.5 μ)
3—septate	40.0 × 3.8 μ	(29.8–51.4 × 3.4–4.5 μ)
4—septate	51.6 × 3.9 μ	(41.5–59.7 × 3.4–4.9 μ)
5—septate	54.6 × 3.9 μ	(48.1–59.7 × 3.5–4.9 μ)

The micronconidia are borne on simple or branched conidiophores, usually in false heads (Fig. 1, D), or singly, but never in chains on any type of substrate. Macronconidia (Fig. 1, B), which are borne on the aerial mycelium, in sporodochia, or pionnotes, are 1- to 5-septate, the 3-septate spores being most abundant.

The ascospores (Fig. 1, A) are straight, slightly constricted at the septa, and short-elliptical in shape. They are predominantly 1-septate, although a few 2-septate spores have been observed. The 1-septate spores measured 12.1–17.0 × 4.5–7.0 μ, averaging 14.8 × 5.6 μ.

The asci are relatively long, narrow, and subclavate in shape (Fig. 1, A). Usually 8 ascospores are found within each ascus; 4- and 6-spore asci are only occasionally found. The asci measured 75–100 × 10–16 μ.



Photographed by J. A. Carlile

FIG. 1. A. 4-, 6- and 8-spore asci. B. Macronconidia. C. Microconidia. D. Micronidia showing the false heads in which they are borne. E. Perithecium. About $\times 120$.

The perithecia, which are practically indistinguishable from those of *Gibberella saubinetii*, measured $270\text{--}350 \times 240\text{--}300 \mu$ (Fig. 1, E). They are borne superficially on corn stalks and occur either scattered or in groups.

Growth on Various Media. Eight single ascospore cultures (B-1 to B-8), all of which were derived from the same ascus, together with 2 isolates

of *Fusarium moniliforme*⁴ (E-1 and Y-29), were grown on 8 different agar media at room temperature. Each culture was seeded in triplicate on each of the 8 media. The pieces of mycelium used to seed the plates were of uniform size and cut from the marginal growth of young colonies that had been grown on the same medium. The diameters of the colonies were measured every 24 hours for a period of 7 days.

In table 1 are shown the diameters of the colonies, at the end of the 7th day, grown on the 8 media. Isolates B-1 and B-8 generally gave the most rapidly-growing colonies of *Fusarium moniliforme* var. *subglutinans* on all media. The two cultures of *F. moniliforme* grew more slowly on most media than did those of *F. moniliforme* var. *subglutinans*; this was especially noticeable on potato-dextrose agar. On Coon's medium agar, however, a marked difference in the general trend of growth rate was observed. Here the two cultures of *F. moniliforme* showed a more rapid rate of growth than did any of the cultures of *F. moniliforme* var. *subglutinans*.

TABLE 1.—Colony diameters in millimeters of 7-day-old cultures of *Fusarium moniliforme* var. *subglutinans* and *F. moniliforme* grown at room temperature on 8 agar media

Agar media	Colony diameters in millimeters									
	F. moniliforme var. subglutinans								F. moniliforme	
	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	E-1	Y-29
Malt	78	70	72	78	70	72	70	81	73	65
Prune	76	73	72	75	75	70	71	75	75	70
Potato-dextrose	90 ⁺ ^a	90 ⁺	90 ⁺	90 ⁺	90 ⁺	90 ⁺	85	90 ⁺	75	70
Oatmeal	78	73	75	75	74	75	74	76	72	65
Cornmeal	82	76	76	76	76	73	72	80	65	65
Lima bean	81	74	75	78	78	68	68	85	68	65
Richard's	76	71	69	73	71	74	73	80	73	73
Coon's	67	65	67	67	65	67	65	67	74	72

^a The + sign indicates that the colonies had grown over the entire surface of the agar.

The colonies listed in table 1, together with single cultures of each of the isolates growing on steam-sterilized rice, were held until 15 days of age, when observations on color production were made. A comparison of the stromatic color between *Fusarium moniliforme* var. *subglutinans* and *F. moniliforme* growing on different media is given in table 2. No appreciable differences were observed in color production between the isolates of *F. moniliforme* var. *subglutinans*, or between the 2 isolates of *F. moniliforme*.

⁴ The cultures of *F. moniliforme* were furnished through the kindness of Miss Helen Johann of the United States Department of Agriculture.

TABLE 2.—*Comparison of the stromatic color of Fusarium moniliforme var. subglutinans and F. moniliforme grown on different media*

Differential media	Color of stroma on various media	
	<i>F. moniliforme</i> var. <i>subglutinans</i>	<i>F. moniliforme</i>
Malt agar	Purplish red ^a	Veronia purple
Prune “	Transparent white	Transparent white
Potato-dextrose “	Blackish-red-purple	Vinaceous purple
Oatmeal “	White	Russet vinaceous
Cornmeal “	Transparent white	Transparent white
Lima bean “	“ “	“ “
Richard's “	Cream buff	Apricot yellow
Coon's “	White	White
Steamed rice	Dahlia carmine	Cream color

^a Colors based on Ridgeway's standards—Ridgeway, R. Color standards and nomenclature. 43 pp. Washington, D. C. 1912.

The most striking difference in color between the two species was brought out on steam-sterilized rice, a critical medium for color diagnosis (10).

Growth at Different Temperatures. One culture of *Fusarium moniliforme* var. *subglutinans*, B-6, and 2 cultures of *F. moniliforme* were grown on potato-dextrose agar at 5, 10, 15, 20, 25, 30, 35 and 40° C. The plates were seeded in triplicate with uniform amounts of mycelium from 7-day-old colonies grown at the same temperatures. In cases of the extreme temperatures, where certain cultures failed to grow, the mycelium used to seed the plates was taken from a colony grown at the next closest temperature. The diameters of the colonies were measured every 24 hours.

In table 3 are given the diameters of the colonies in millimeters at each temperature at the end of 6 days. The isolate of *Fusarium moniliforme* var.

TABLE 3.—*Colony diameters at the end of the 6th day of Fusarium moniliforme and F. moniliforme var. subglutinans grown on potato-dextrose agar at 8 different temperatures*

Isolate	Colony diameters (in mm.) at different temperatures							
	5°	10°	15°	20°	25°	30°	35°	40°
E-1, <i>F. moniliforme</i>	7	20	37	55	85	90	31	0
Y-29, <i>F. moniliforme</i> ...	0 ^a	17	34	38	72	75	16	0
B-6, <i>F. moniliforme</i> var. <i>subglutinans</i>	11	25	41	57	82	77	8	0

^a The slight growth occurring here was not measurable.

subglutinans, B-6, showed a markedly lower growth-temperature range, with a minimum below 5° C., an optimum at about 25° C., and a maximum between 35° and 40° C. The isolates of *F. moniliforme* showed less growth below 20° C. and a somewhat higher optimum of about 30° C. Although the maximum temperature for growth is approximately the same for *F. moniliforme* var. *subglutinans* and *F. moniliforme*, the latter is able to grow considerably better at 35° C.

Pathogenicity. In order to determine the pathogenicity of the fungus, kernels of yellow dent corn were planted in rich compost soil that had been infested with cornmeal-sand cultures of the fungus at the rate of 4 parts of soil to 1 part of culture. Control plantings were made in soil mixed in the same proportion with sterile cornmeal-sand medium. The experiment was carried out in a greenhouse where a soil temperature of about 22° C. was maintained.

When the control seedlings had reached the 3-leaf stage, 10 days after planting, all seedlings were removed from the soil, washed clean, and examined for disease. Over 80 per cent of seedlings in the inoculated lots showed varying degrees of blighting, while those of the control plantings showed less than 10 per cent disease. From the inoculated seedlings *Fusarium moniliforme* var. *subglutinans* was readily reisolated, whereas the diseased seedlings from control plantings gave rise to *Penicillium* sp., *Aspergillus* sp., and an undertermined *Fusarium* sp.

In general, the symptoms of corn seedling blight brought about by infection with this fungus were very similar to those caused by *Fusarium moniliforme* (8). A marked delay in emergence, followed by a retardation in growth, was the first indication of infection. The root systems of inoculated kernels were reduced in extent (Fig. 2) and marked with numerous reddish-brown lesions. Lesions of varying size were found centering around points where primary and adventitious roots break through coleorhiza and mesocotyl, respectively.

Sexuality. To determine if sex groups existed within the variety, a number of experiments were conducted in which single cultures, and cultures paired in all possible combinations, were grown on different substrates.

In one experiment, 8 cultures, each of single ascosporic origin and derived from a single ascus, were grown individually and in all possible pairings on plants of potato-dextrose agar. At the end of 2 months, at which time the experiment was discontinued, there was no indication of perithecia in any of the cultures.

A second experiment was conducted in which 9 cultures, 8 single and 1 a composite of all 8, were grown on plates of ground whole-oat agar. After a lapse of 2 months no perithecia were observed in the cultures.



FIG. 2. The 3 seedlings at the left are healthy. The 7 seedlings at the right show all degrees of infection with *Fusarium moniliforme* var. *subglutinans*.

In a third experiment, 9 cultures, 8 single and 1 a composite of all 8, were grown on sterilized whole oats in 250-cc. Erlenmeyer flasks. After a period of 1 week these cultures were placed in separate earthenware pots containing sand, covered with cheesecloth, and removed to the greenhouse. The pots were set far apart and precautions were taken to prevent contamination of one culture by another. Within 3 weeks after placing the cultures in the greenhouse, during which time they were watered daily, mature perithecia

had formed in cultures B-5 and B-8, and in the composite culture of all 8 isolates. A number of the cultures produced an abundance of perithecia, but the latter remained small and never reached maturity. When the experiment was repeated, essentially the same results were obtained, with the exception that a longer time (5 weeks) elapsed before mature perithecia were observed in culture B-5.

These experiments indicate that the fungus is homothallic and that some cultures, originally derived from single ascospores, are able to produce mature perithecia if given the proper environmental conditions. Other cultures of similar origin, but perhaps different physiologically, are unable to develop mature perithecia under the same conditions.

DISCUSSION

Fusarium moniliforme var. *subglutinans* and *F. moniliforme* are the only members of the section *Liseola* of the genus *Fusarium* in which an ascigerous stage is known. Furthermore, their perfect stages, namely, *Gibberella fujikuroi* var. *subglutinans* and *G. fujikuroi*, are the only known members of the genus *Gibberella* in which 1-septate ascospores are predominant.

In the present studies the measurement of the spore forms, together with other diagnostic characters, correspond very closely to those given by Wollenweber and Reinking (9). In view of the above facts, along with differentiating characters brought out in the experiments reported in this paper, there appears to be little doubt as to the identity of this fungus.

It is probable that a wider distribution of the fungus will be found in this country than indicated in the present paper. The question as to whether it is endemic to the United States and has been overlooked or mistaken for *Gibberella fujikuroi*, or has recently been introduced into this country, is difficult to answer. In spite of the possibility of confusion between the species and its variety, *subglutinans*, the most plausible explanation for the occurrence of the variety in the United States seems to be that of recent introduction.

SUMMARY

The occurrence of *Gibberella fujikuroi* var. *subglutinans* in the United States is reported and descriptions of its morphology and cultural characters are given.

The conidial stage of the fungus could be differentiated from *Fusarium moniliforme* by its faster growth rate and more intense coloration on different media, particularly on steamed rice.

The minimum and optimum temperatures for growth of the varietal form were somewhat lower than those of the type species, *F. moniliforme*.

Certain cultures of single ascospore origin were shown to be capable of producing mature perithecia, indicating that the fungus is homothallic.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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CHEMICAL CONTROL OF HARMFUL FUNGI DURING STRATIFICATION AND GERMINATION OF SEEDS OF *RIBES ROEZLI*

CLARENCE R. QUICK

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INTRODUCTION

The research results here reported were obtained under the authority of the Division of Blister Rust Control, while it was yet in the Bureau of Plant Industry, U. S. Department of Agriculture.¹

In carrying out a program of research² on the physiology and morphology of plants of the genus *Ribes* L., currants and gooseberries, it has been necessary to grow large numbers of seedlings for experimental use. The seeds of most *Ribes* species germinate most satisfactorily after several months of moist stratification at low temperatures. Preliminary work on the routine propagation of *Ribes* plants showed that damping-off fungi invariably caused a high seedling mortality during the extended period of stratification and germination. The experiments summarized in this paper represent a study of practical methods for controlling damping-off fungi in *Ribes* seed cultures during stratification and germination.

EXPERIMENTAL PROCEDURE

The seeds of *Ribes roezli* Regel used in the experiments were collected in the fall of 1930 at an elevation of about 6,300 feet, in the Stanislaus National Forest of California. They were stored as air-dry seeds at room temperature for 1 year, and air-dry at 5° C. for an additional year. The seeds were in excellent condition when planted in the cultures at the beginning of the tests. Two series of cultures were run.

In the first series of cultures, 65 paraffined paper cups were filled to within $\frac{1}{2}$ inch of the top with a mixture of equal parts river sand, fine forest loam, and Sphagnum moss which had been passed through a 12-mesh sieve. The cups held about 105 ml. of soil, had a soil surface of about 24.5 sq. cm. (3.8 sq. in.), and required about 60 ml. of water or chemical solution to saturate the propagating medium. The sand had been used in previous experiments and was known to be heavily infested with damping-off fungi. Sixteen reagent chemicals³ were selected for the tests, and were applied

¹ Effective December 1, 1933, the Blister Rust Control work was transferred to the Bureau of Entomology and Plant Quarantine.

² This work, part of an investigative program on the chemical eradication of *Ribes*, has been made possible through the active cooperation of the Department of Botany and the College of Agriculture of the University of California at Berkeley.

³ By the term "reagent chemicals" the writer signifies those chemicals commonly kept in stock in a laboratory.

in the manner best adapted to their chemical properties. Thus, liquid chemicals were diluted with water and used to saturate the soil of the culture; and solids were applied (a) in aqueous solution, (b) as a dry powder, or (c) suspended in water. All treatments, except those in which the seeds were dusted with dry powder, were made immediately after the seeds were planted in the cultures. Each culture consisted of 40 seeds of *Ribes roezli* planted at a depth of about $\frac{1}{2}$ cm.

The 65 cultures⁴ were placed in 4 small flats, covered with paraffined paper and subjected to a period of $3\frac{1}{2}$ months of moist stratification at $21\frac{1}{2}^{\circ}$ C. In April, 1933, the flats were removed to a greenhouse for germination test.

A second set of 35 cultures⁵ was started in October 1933, using seeds of the same collection. The same type of propagating medium and the same period of stratification at $21\frac{1}{2}^{\circ}$ C. were employed. To reduce crowding of the seedlings, larger paraffined cups were used than in the first set of cultures. These larger containers held about 120 ml. of soil, had a soil surface of about 42 sq. cm. (6.5 sq. in.), and required about 75 ml. of solution to saturate the propagating medium.

The most satisfactory seedling production was obtained by the use of copper oxalate, basic copper carbonate, formaldehyde, and nitric acid. As compared with an average of 5 per cent of seedlings satisfactory for propa-

⁴ A complete list of cultures of the first set is given below. Controls 3. Seeds dusted with basic cupric carbonate powder, and with anhydrous cupric sulphate. Soil treated with flowers of sulphur at rates of 2, 4, 8, and 16 grams per 946 ml. (1 qt.) of soil and with chlorinated lime at rates of 2, 4, and 8 grams per 946 ml. soil. Cultures treated with basic cupric carbonate powder at rates of $\frac{1}{2}$, 1, 2, 4, and 8 grams per 929 sq. cm. (1 sq. ft.) of surface; with cupric oxalate at the same rates; and with mercurous chloride at rates 0.05, 0.10, 0.15, and 0.20 grams per 929 sq. cm. Glacial acetic acid was applied to cultures in dilutions (by volume) of 1:500, and 1:1000. Formaldehyde, 40 per cent, 1:400, 1:600, and 1:800. Sulphuric acid, 1:250, 1:500, and 1:1000. Nitric acid, 1:250, 1:500, and 1:1000. Sodium hypochlorite, 5 per cent solution, 1:125, 1:250, 1:500, and 1:1000. Hydrogen peroxide, 3 per cent, 1:5, 1:11, and 1:23. Mercuric chloride was dissolved in water and applied to cultures in dilutions (by weight) of 1:500, 1:1000, 1:2000, and 1:4000. Aluminum sulphate (hydrated) 1:62.5, 1:125, 1:250, 1:500, and 1:1000. Cupric sulphate (hydrated) 1:250, 1:500, 1:1000, and 1:2000. Sodium chlorate, 1:500, 1:1000, 1:2000, 1:4000, and 1:8000. Boric acid, 1:500, 1:1000, and 1:2000.

⁵ The complete list of cultures of the second set follows. Controls 2. Seeds were dusted with basic cupric carbonate. Cultures were treated with basic cupric carbonate at the rates of 2, 3, 4, 6, and 8 grams per 929 sq. cm. (1 sq. ft.); and with cupric oxalate at rates of 3, 6, 9, and 12 grams per 929 sq. cm. Glacial acetic acid was applied to cultures in dilutions (by volume) of 1:500, 1:1000, and 1:1500. Formaldehyde, 40 per cent, 1:750, 1:1000, and 1:1250. Hydrogen peroxide, 3 per cent, 1:2. Nitric acid, specific gravity 1.42, 1:125, 1:200, 1:250, 1:350, and 1:500. Sulphuric acid, specific gravity 1.84, 1:500, 1:1000, and 1:2000. Sodium hypochlorite, 5 per cent solution, 1:1000 and 1:2000. Aluminum sulphate (hydrated) was dissolved in water (by weight) and applied in dilutions of 1:15, 1:30, and 1:60. Copper sulphate (hydrated) 1:125, 1:250, and 1:500.

gation in the untreated cultures at the end of the test, the copper oxalate cultures averaged 42 per cent, the formaldehyde 34 per cent, the nitric acid 29 per cent, and the copper carbonate 24 per cent. Table 1 presents for comparison the results of some of the more satisfactory tests.

TABLE 1.—Percentage production of seedlings in some of the more satisfactory cultures

Chemical used	Dilution of substance with water	Grams per 929 sq. cm. (1 sq. ft.) soil surface	First set cultures		Second set cultures	
			Satisfactory seedlings in culture at end of test	Total seedlings produced	Satisfactory seedlings in culture at end of test	Total seedlings produced
Water (controls)	3	11	6	39
Copper carbonate						
$\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$, C. P.	2	28	70
do	3	6	44
do	4	38	78
do	6	14	80
do	8	23	100	36	56
Copper oxalate						
$\text{Cu}(\text{COO})_{2\frac{1}{2}} \cdot \text{H}_2\text{O}$, C. P.	3	26	56
do	4	18	63
do	6	64	70
do	8	50	98
do	9	56	64
do	12	40	74
Formaldehyde						
HCHO , 40%, U. S. P.	1:600	20	40
do	1:750	74	98
do	1:800	30	58
do	1:1250	12	92
Nitric acid						
HNO_3 , 70%, C. P.	1:200	22	70
do	1:250	58	80	10	90
do	1:500	50	70	4	98

Finely powdered cupric oxalate gave good protection to seeds and seedlings when applied to cultures at rates of 6 to 10 grams of chemical per 929 sq. cm. (1 sq. ft.) of soil surface. Its protection lasted throughout the $3\frac{1}{2}$ months of moist stratification at $2\frac{1}{2}^\circ \text{C}$., and a subsequent 6 weeks' germination test in the greenhouse. Finely powered basic cupric carbonate was found satisfactory, but slightly less effective than cupric oxalate. The best rates of application were 4 to 8 grams per 929 sq. cm. The most convenient methods of applying the insoluble powders were: (1) to distribute the dry

powder evenly over the surface of the culture and then to wet down with a florist's syringe, or (2) to suspend the powder in water and to apply to the surface of the culture. Since the completion of the experimental work, copper oxalate and copper carbonate have been used repeatedly and successfully for the control of damping-off fungi in cultures of a wide variety of *Ribes* seeds. The use of these two compounds on seed cultures of other plants, such as snapdragon, petunia, etc., has consistently protected the seedlings from damage by fungi.

Dilute nitric acid and formaldehyde protected the seeds fairly well during stratification (the former causing a marked acceleration of seedling growth), but, presumably because of high reactivity and consequent rapid disappearance, gave less sure protection than the relatively insoluble copper compounds. In the nitric acid and formaldehyde series, best results were secured with a solution of 1 part of 70 per cent nitric acid to 250 parts of water and with 1 part of commercial 40 per cent formaldehyde to 750 parts of water.

FEEDING HABITS OF THE NEMATODES APHELENCHOIDES PARIETINUS AND APHELENCHUS AVENAE

J. R. CHRISTIE AND C. H. ARNDT

(Accepted for publication Sept. 23, 1935)

It appears to be the prevalent opinion of workers in the field of free-living and plant-parasitic nematodes that *Aphelenchoides parietinus* (Bastian, 1865) Steiner, 1932 and *Aphelenchus avenae* Bastian, 1865 are saprophytes. Thorne¹ refers to *Aphelenchus avenae* as a saprophytic species and Goodey² regards both *Aphelenchoides parietinus* and *Aphelenchus avenae* as "mainly saprophytic in habit." The latter investigator cites this saprophytic habitat as evidence possibly indicating that the stylet of plant-infesting members of the Anguillulidae may not necessarily be "a puncturing or offensive organ." In this connection Goodey goes on to say, "Again, there seem to be no records of direct observations of the stylet actually functioning as a puncturing organ. Admitting that such evidence may be extremely difficult to obtain, it is surely not without significance that no one has, so far, produced it." This being the case, observations by the writers on the habits of *Aphelenchoides parietinus* and *Aphelenchus avenae* appear worthy of record.

Both *parietinus* and *avenae* can be reared on agar plates. A cornmeal nutrient agar on which the fungus *Neurospora sitophila* is growing has been found satisfactory and was used by the writers in their experimental work with these nemas. While attempting to determine the most suitable culture medium it was noted that *avenae* and *parietinus* developed and reproduced only when the plate was contaminated with a fungus. It was further noted that the nemas frequently gather near the periphery of a fungus colony. By using a comparatively transparent agar and pouring thin plates it is possible to place the preparation on the stage of a microscope and observe the specimens under a magnification of $\times 150$. An individual that has penetrated the agar and is located near the bottom of the Petri dish is more satisfactory for study, as the agar retards movement and the preparation can be inverted and the nema viewed through the bottom of the dish. Under these conditions the feeding habits of these nemas can be distinctly seen. After the head is placed against a hypha there is a brief but rapid backward and forward movement of the stylet followed immediately by an equally rapid muscular movement of the bulb. Granules and other cellular inclusions in the hypha can be seen moving towards the point where the

¹ Thorne, Gerald. The nematode *Neotylenchus abulbosus* Steiner (Anguillulidae) as a parasite of sugar-beets. Helminthol. Soc. Wash. Proc. 2: 46. 1935.

² Goodey, T. The pathology and etiology of plant lesions caused by parasitic nematodes. 34 pp. Imp. Bur. Agr. Parasitol., St. Albans, Eng. 1935.

stylet is inserted. The stylet did not remain appreciably protruded while the contents of the hypha were being removed. These observations apply both to *parietinus* and *avenae* and leave little doubt as to the source of food of these

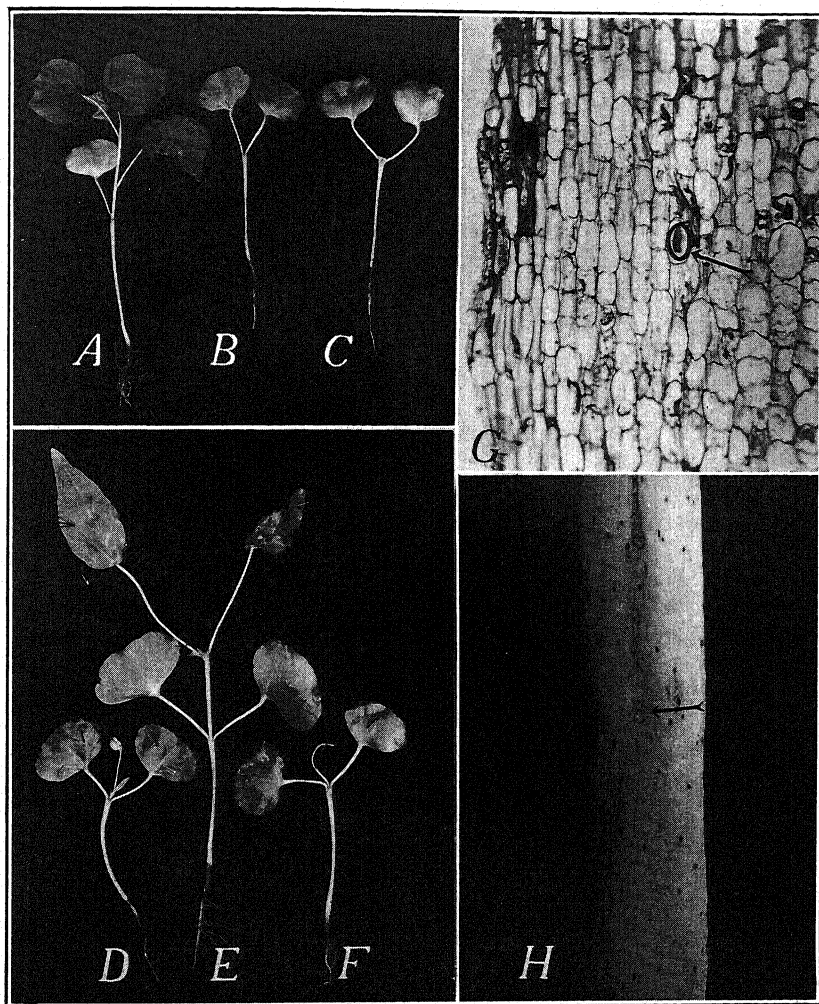


FIG. 1. Cotton seedlings attacked by the nematode *Aphelenchoides parietinus*. A. Normal seedling. B to F. Seedlings the terminal buds of which are infested with *A. parietinus*. These seedlings, grown at a constant soil temperature of 21° to 22° C., required 7 to 9 days for germination. G. Longitudinal section through cotton hypocotyl showing specimen of *A. parietinus* within cortical cell and surrounded by apparently healthy tissue. The diseased tissues of a shallow lesion show on the upper left side of the photograph. H. Cotton hypocotyl showing small pit presumably caused by *A. parietinus* and from which three living specimens of this nema were removed.

nemas when they are growing on culture media. In nature parietinus and avenae are frequently found associated with decaying organic matter. However, this is likewise a favored environment for many fungi and it seems probable that the nemas are attracted by such fungal growth.

There is considerable evidence that fungi are not the only source of food. Both parietinus and avenae are frequently found associated with lesions on the underground parts of plants, especially on the hypocotyls of seedlings. Sometimes these nemas migrate beyond the diseased area, penetrating apparently healthy cortical tissue, and may be found coiled within cells (Fig. 1, G) or in intercellular spaces. In such instances it seems reasonable to suppose that they are utilizing the contents of these cells as food.

In connection with investigations regarding the cause of lesions on the hypocotyls of cotton seedlings the writers have carried on a series of greenhouse experiments growing the seedlings in soil experimentally infested with a species of nematode or a species of fungus or a combination of a nematode and a fungus. The soil was first steam-sterilized and the nematodes and fungi used for its subsequent inoculation were both culture-reared. This work is still in progress and complete results will be published later, but certain observations may appropriately be recorded in connection with the present discussion.

One experiment involved 25 lots of seedlings, 5 lots of which were grown in soil infested with parietinus. When the seedlings were removed and the roots washed it was noted that, in these 5 lots from parietinus-infested soils, the roots had a yellowish to brownish color, sufficiently pronounced to readily distinguish them from the other 20 lots. The explanation appeared to be that parietinus had been feeding on the epidermal cells of the roots.

In these and similar experiments with cotton seedlings, parietinus sometimes infested the terminal bud. This infestation undoubtedly took place before the seedling broke through the soil and was carried up as the plant grew. It occurred when germination took place at constant soil temperatures of 21° C. and below. It was not observed when the cotyledons emerged from the soil within 5 days after the time of planting. Infested plants are sometimes "blind," failing to produce secondary leaves (Fig. 1, B and C) or, if secondary leaves develop, they may be crinkled (Fig. 1, E) or small and distorted (Fig. 1, D and F) according to the degree of injury to the terminal bud. On dissecting the terminal buds of these abnormal plants from 1 to 10 specimens of parietinus per bud were found among the bases of the leaf primordia; never definitely internal to the epidermis. In such instances parietinus behaves very much like *Aphelenchoides fragariae* on strawberries.

Small pits frequently occur on the hypocotyls of cotton seedlings, which, on microscopical examination, are found to harbor specimens of parietinus

(Fig. 1, H). These pits are apparently of mechanical origin, and one feels justified in concluding that they are produced by the nemas. A previously reported experiment by the writers³ furnishes further evidence that nemas can and do produce such pits. In this experiment nemas [mostly *Panagrolaimus* sp. (= *Cephalobus* of authors, in part)] were placed on or near the hypocotyl of a cotton seedling growing in sterile agar. Sixteen hours later 6 small pits had been produced in which nemas were found.

When cotton seedlings were grown at a constant soil temperature of 18 to 19° C. requiring 12 to 15 days for germination, there sometimes persisted on the cotyledons yellowish brown areas that did not turn green as normal tissue. Living specimens of *parietinus* were found in these areas. This occurred only on seedlings grown in soil inoculated with *parietinus*.

Observations on cotton seedlings indicate that *parietinus* and *avenae* may attack diseased plant tissues and perhaps utilize as food the contents of cells in early stages of necrosis. In such instances these nemas may conceivably be regarded as saprophytic. Even so the contents of living plant cells constitute part, and probably the greater part, of their food. The importance of these nemas as pathogenic agents is difficult to determine. Either alone or in conjunction with certain fungi they may be of considerable significance in initiating or perpetuating some of those root troubles, the causes of which have been so obscure and perplexing. While the writers' endeavors to determine experimentally the rôle of these and other nemas in the etiology of cotton sore shin have been disappointingly inconclusive, nevertheless neither *Aphelenchoides parietinus* nor *Aphelenchus avenae* can yet be dismissed as merely saprophytic secondary invaders of no significance.

BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.,
AND SOUTH CAROLINA AGRICULTURAL EXPERIMENT STATION,
CLEMSON COLLEGE, S. C.

³ Christie, J. R., and C. H. Arndt. Further notes on the nematodes associated with the sore shin of cotton. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rpt., 17: 10-12. 1933. [Mimeog.]

OVERWINTERING OF ERWINIA AMYLOVORA IN ASSOCIATION WITH SEVERE WINTER INJURY ON BALDWIN APPLE TREES

E. M. HILDEBRAND

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The unusually low temperatures of the winter of 1933-34, according to surveys made by R. L. Gillett,¹ Agricultural Statistician of New York (1934), killed outright in New York alone 1,361,000 apple trees of bearing age and left more than 2,000,000 trees crippled. Heavy losses were sustained also by the growers of other fruits, with peaches, sweet cherries, and quinces suffering most.

The presence of winter injury in blight-susceptible orchards altered considerably the winter-pruning program ordinarily consisting of the removal of fire-blight cankers followed by the shaping of the trees. To provide the trees with a maximum of foliage for recovery, pomologists were recommending that pruning operations be suspended until such time during the growing season as would indicate the condition of injury of the trees. This suggestion raised the question of the possible disease hazard from the omission of canker removal. In case the cankers were to be removed it introduced to the grower the difficult problem of distinguishing between canker injury and cold injury.

Accordingly, the effect of winter injury on the incidence of blight was studied in two of the writer's experimental orchards of Bartlett pear and Baldwin apple where there had been severe epiphytotics of fire blight in 1933. The plant-tissue injury in the Bartlett pear orchard was so severe that the bark on many of the trees was blackish, whereas in the Baldwin apple orchard, although considerable sapwood was discolored, the principal injury to the bark was in association with the canker margins. In cases of such diversity it was thought that a bacteriological study of the survival of the fire-blight organism, *Erwinia amylovora*, in the pear and apple cankers in these orchards would help to clarify the situation.

In March, after the last February cold wave, a number of fire-blight cankers from the Bartlett pear orchard of Ralph Nelson at Clyde, N. Y., were brought into the laboratory for study. The injury was so extreme that the discoloration extended throughout the tissues in the majority of the trees examined. This resulted later in the death or severe crippling of a large

¹ N. Y. State Dept. of Agr. and Markets, Bureau of Statistics. Winter damage to fruit trees and grape vines, winter of 1933-34 (Preliminary). In cooperation with U. S. Dept. of Agr., Bureau of Agr. Economics, Div. of Crop and Livestock Estimates. 9 numb. leaves. 1934. [Mimeographed.]

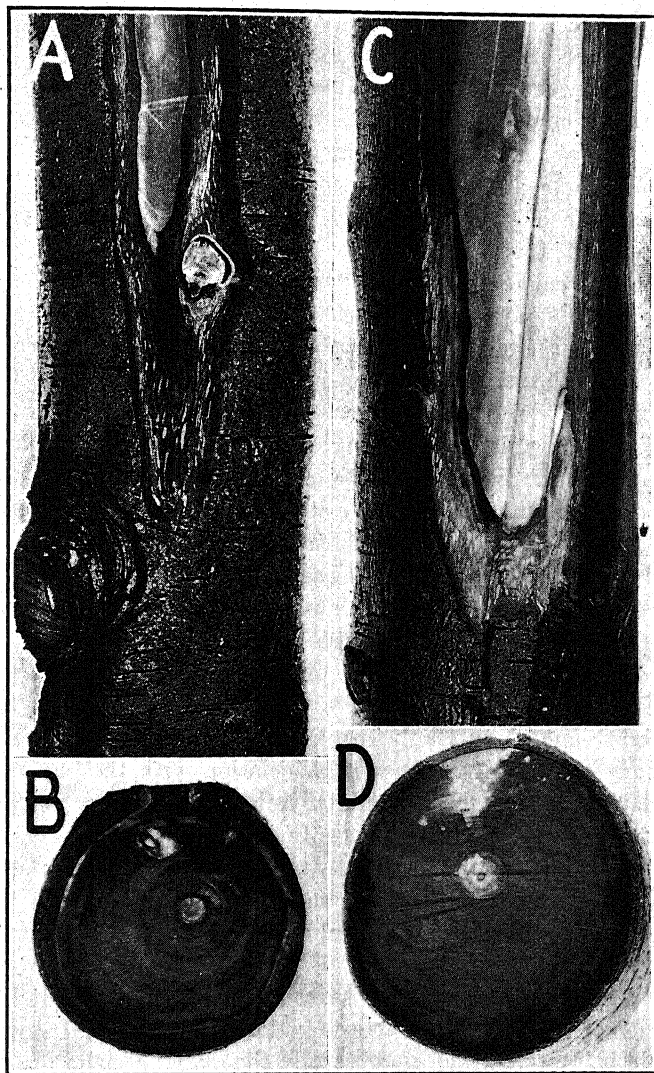


FIG. 1. Winter injury in Bartlett pear. A. Showing severe winter injury to a limb 3 inches in diameter. A cut was made to expose the tissues of the bark, which were blackish and interspersed longitudinally with characteristic light-color streaks. B. Cross section of the limb shown in A. A discontinuous band of light-color tissue in the region of the cambium contained living cells. C. Showing severe winter injury to a blighted limb 3 inches in diameter. The bark from over the canker margin had been cut away to expose the underlying tissues. The characteristic light streaks in black of winter injury are illustrated on the left and on the right is the characteristic reddish brown blighted tissue. D. Cross section of the cankered limb shown above in C. The wood underneath the tip of the canker was apparently normal in color in strong contrast to the dark wood beneath the winter-injured bark.

proportion of the trees. A severely injured pear limb is shown in figure 1, A. The surface of the bark had a blackish cast. When a portion of the bark was cut away the black discoloration was observed to extend to the cambium and throughout the sapwood. In figure 1, B a cross section of the same limb is shown. Only a small layer of tissue located in the region of the cambium appeared alive. Microscopic examination of the cells revealed that the majority of them were discolored, plasmolysed, and apparently dead. In figure 1, C extremely severe winter injury is shown in association with a fire-blight canker. The bark was cut away at the margin to expose the winter-injured and blighted tissue. The winter-injured region on the left shows the characteristic black with many light-color streaks, while the canker tissue on the right shows the usual reddish brown but with fewer light-color streaks. The wood underneath the tip of this canker was almost normal in color (Fig. 1, D) in strong contrast to the dark wood below the winter-injured bark. When cut with a knife the winter-injured tissues in the region of the cambium extruded a watery fluid, indicating that the cells were dead and their membranes had ceased to function.

Attempts to isolate the fire-blight bacteria from 10 cankers on pear gave only negative results. Tissue was plated from 5 different locations around the margin of each canker. Although this study was limited in scope, it appears that the bacteria were unable to survive in cankers under these conditions.

Fire-blight cankers on Baldwin apple trees observed in March showed serious injury of the bark tissues in association with the canker margins. For a variable distance, usually under 1 inch, extending away from the canker margin, the bark tissue was deeply discolored. Moreover, the tissues of the woody cylinder, and particularly of the sapwood, were affected. The discoloration was variable in degree and extent and often ran throughout the bark, being interspersed with numerous light-color streaks typical of winter injury.

Since the winter-injured bark tissues coincided approximately with the usual location of the fire-blight bacteria in dormant cankers,^{2,3} it was thought that the cold injury would affect adversely the overwintering of the bacteria and possibly prevent resumption of canker activity in the spring. When the apple bark tissues were observed microscopically some of the discolored cells showed plasmolysis and appeared to be dead, while others appeared to be alive. Still other cells were not even discolored. The fact that not all of the cells were dead precludes the possibility of knowing the effect on the bacteria in apple bark tissue in which all the cells were killed. However,

² Jones, D. H. Bacterial blight of apple, pear, and quince trees. Ontario Dept. Agr. Bull. 176. 1909.

³ Brooks, A. N. Studies of the epidemiology and control of fire-blight of apple. *Phytopath.* 16: 665-696. 1926.

the case of the Bartlett pear might give some evidence on this point. Except for the already mentioned preliminary isolations from Bartlett pear cankers, all of which were negative, the isolations herein reported were limited to the Baldwin apple trees, all of which survived the winter.

MATERIALS AND METHODS

Isolations were attempted from 345 Baldwin apple cankers from stems ranging between $\frac{1}{4}$ and 4 inches in diameter. The cankers were removed in March, brought into the laboratory and stored in a 32° F. incubator until the isolations were made. Depending on the size of the limb, 2 to 5 isolations were attempted from each canker. The isolations were made from a band of tissue approximately 1 inch wide outside the canker margin. Several samples were taken from over this range.

The isolation technique consisted of, viz., the disinfestation of the surface of the canker with bichloride of mercury (1-1000), the removal of the exposed outer bark by making cuts back and forth with a sterile scalpel, and the transfer aseptically of cubes of tissue to sterile water in Petri dishes. The cubes were chopped fine with a sterile scalpel and allowed to stand for about 10 minutes before making loop dilutions to 3 successive plates. The plates were poured with nutrient agar and incubated at 24° C. Observations were made at daily intervals for growth and the bacterial colonies appearing were transferred to nutrient agar slopes. A 2-day-old growth on agar was used for pathogenicity tests. Inoculation was by needle puncture into the receptacle cups of flowers of dwarf Bartlett pear forced in the greenhouse. The isolates from different cankers were never inoculated into the flowers of the same cluster. At least one flower in a cluster was left as a check. Observations were made at daily intervals. Positive symptoms or the presence of droplets of ooze usually appeared from $2\frac{1}{2}$ to 4 days after inoculation. The blossoms showing positive symptoms were removed with scissors and stored. Reisolations and re inoculations were made with doubtful cultures.

EXPERIMENTAL

The results of the isolation studies summarized in table 1 provide evidence that the bacteria were able to survive in winter-injured apple tissue. The fire-blight bacteria were recovered from 85 or 24.6 per cent of the 345 cankers examined. From a total of 1061 isolation attempts the organism was recovered 156 times or in 14.7 per cent of the cases. All sizes of cankers yielded the causal organism except cankers on limbs over 3 inches in diameter. In this size group there were but 3 cankers. Of the cankers examined 152, or 44.1 per cent, were apparently sterile.

From the other 193 cankers, in addition to the 156 isolates of the causal bacteria, there were 258 cultures of nonpathogenic bacteria. Of these, 91

TABLE 1.—*Results of attempts to recover the fire-blight organism from dormant cankers on winter-injured Baldwin apple trees*

	Diameter in inches of the cankered limbs					
	$\frac{1}{4}$ – $\frac{1}{2}$	$\frac{1}{2}$ –1	1–2	2–3	3–4	Total $\frac{1}{4}$ –4
Cankers examined ...	6	210	104	22	3	345
Cankers alive	1	51	28	5	0	85
Per cent alive	16.7	24.3	26.9	22.7	0.0	24.6

simulated the fire-blight organism, 26 were white filiform, 33 white spreading, 28 gray to cream color, 50 yellow to orange, and 30 miscellaneous. The exact significance of the secondary invaders has not been determined, but it is possible that at least certain of them may antagonize the fire-blight organism.

The trees from which the cankers were removed during the dormant season were reexamined in the fall of 1934 for evidence of what had happened to the cankers that had escaped the eyes of the operators or that had been left for other reasons (Table 2). The bulk of these cankers had smooth

TABLE 2.—*Results of field examinations in the fall of 1934 for evidences of activity of fire-blight cankers on winter-injured Baldwin apple trees*

	Diameter in inches of the cankered limbs					
	$\frac{1}{4}$ – $\frac{1}{2}$	$\frac{1}{2}$ –1	1–2	2–3	3–	Total
Cankers examined ...	3	5	32	34	42	116
Cankers alive	0	0	15	15	25	55
Per cent alive	0.0	0.0	46.8	44.1	59.5	47.4

margins, a fairly reliable criterion that many of them were alive (Brooks, 1926). A total of 116 cankers were examined for evidence of spread of infection and 55, or 47.4 per cent of them, had apparently moved 1 to 5 inches during the season. This was determined by cutting away the outer bark and examining for winter-injured tissue, a condition that had been quite generally observed in association with the margins of the cankers while dormant. The active cankers showed the usual tissue symptoms beyond the discolored regions. Puncturing the advancing margins with a needle or nail resulted in the issuing forth from the wound of a milky fluid that contained the bacteria in great abundance. Some of these cankers were alive and exuding during the summer of 1935.

DISCUSSION AND CONCLUSIONS

The fact is well known that the fire-blight organism overwinters in a narrow band of tissue outside the canker margin. Although the winter injury to the bark adjoining fire-blight cankers coincided with the usual location of the causal bacteria, it may be concluded from these studies that it does not prevent the bacteria from surviving the winter. Where nearly all the cells were killed, as in the case of Bartlett pear, it was anticipated that the bacteria would die as they ordinarily do in the dead tissue of cankers. In the apple tissue, however, not all of the cells were dead at the time of the examination. The presence of living cells may account for the apparent lack of effect on the bacteria.

A complicating factor, evident at once to those who have studied this disease, is the extreme variability in the percentage of cankers that survive the winter under more normal conditions on the same or different varieties of trees. On the basis of earlier observations on the overwintering of cankers in Baldwin apple trees it appears that a much larger percentage survived in association with the winter injury. It seems that the presence of the bacteria or of the canker tissue increased the susceptibility of the adjacent apparently healthy tissue to cold injury and the cold-injured tissue in turn was apparently more favorable as a medium for the survival of the bacteria. Pomologists are well agreed that any influence that depletes the supply of nutrients to the tissues in fruit trees has a tendency to weaken their resistance to adversity and especially to cold weather conditions.

The decreased vitality of the winter-injured trees seemed to hold the disease in check, which explains the small amount of advance made by the cankers during the subsequent growing season. A further contributing factor was an extremely dry spring season in 1934 during which no oozing cankers were observed in the block of Baldwin trees.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY.

PHYTOPATHOLOGICAL NOTE

Galls Produced by Plant Hormones, Including a Hormone Extracted from Bacterium tumefaciens.—It has been demonstrated by various workers¹ that plant growth hormones will, in many plants, produce a bending of stems, roots, and petioles, inhibit bud development, speed up callus formation following bud decapitation, and stimulate root production in cuttings. The work reported in this paper adds to the above list the production of galls.

The growth substances used for gall production were indoleacetic² and indolepropionic acids. They were used in a salve in which 20 milligrams of the growth substance were mixed with one gram of lanolin. The lanolin mixtures were applied to decapitated stems and side wounds on stems as a film smear, completely covering the wound. By another method, stems were smeared without wounding.

The experimental plants were beans, tobacco, tomato, sunflower, privet, Paris daisy, *Bryophyllum pinnatum*, *Kalanchoe daigremontiana* and *Impatiens balsamina*.

Galls were produced by smearing the wounded stems with a film of the mixture. Only one application was necessary. (Figs. 1 and 2).³ It might be stated here that in the production of bacterial and fungus galls, occurring either naturally or by artificial inoculation, a lesion is necessary for the entrance of the organism. Bending of stem or petiole may occur in a few hours by the mere application of the growth substance to the uninjured epidermis, but it appears that a definite wound is necessary for gall production. Bean, tobacco, and sunflower plants were the most satisfactory used in these experiments for gall production, although Paris daisy, privet, and *Impatiens balsamina* also produced definite galls. *Bryophyllum* and *Kalanchoe* responded by forming a few roots. *Ricinus*, though tried repeatedly, showed no trace of root or gall formation. Tobacco (Fig. 2, B) and sunflower produced galls rather slowly. None of the hormone galls developed so rapidly as did those of a *Bacterium tumefaciens* gall growing on a favorable host. However, in the case of the bean, the galls produced by a very virulent culture of *Bacterium tumefaciens* (Fig. 4, B and C) never reached the size of the galls produced by indoleacetic acid on bean (Fig. 1).

¹ No attempt will be made in this brief note to enumerate the workers on plant hormones. For a starting point on the literature of this subject, readers are referred to a fairly recent summary by F. W. Went in Botanical Review 1: 162-182. 1935.

² The indoleacetic acid was obtained through the courtesy of Dr. F. W. Went of the California Institute of Technology, and Dr. N. L. Drake of the University of Maryland. It can now be obtained from reputable chemical firms.

³ One-half the cost of publishing the illustrations accompanying this paper was borne by the authors.

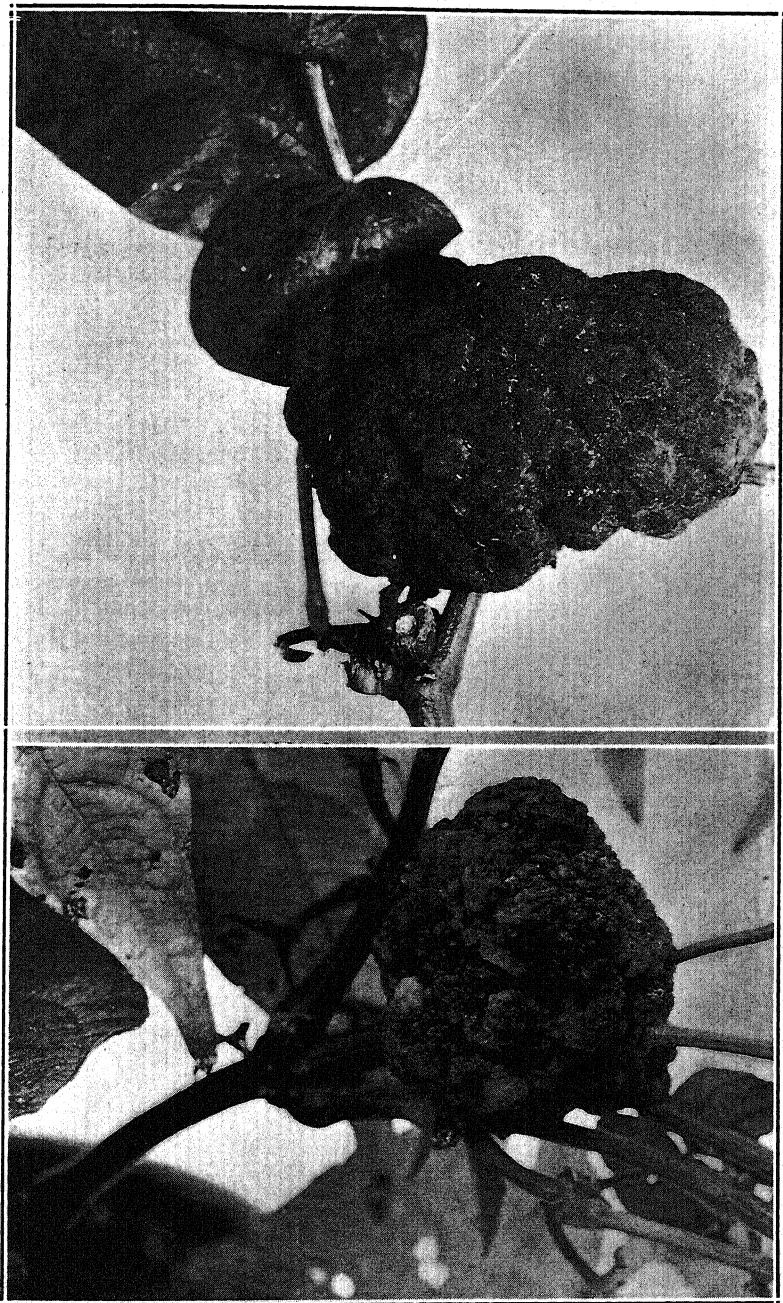


FIG. 1. Galls, produced on Red Kidney bean plants by decapitating and smearing the wound with indoleacetic acid (hetero-auxin) November 18, 1935. Photographed April 25, 1936, while galls were still growing. A little less than natural size.

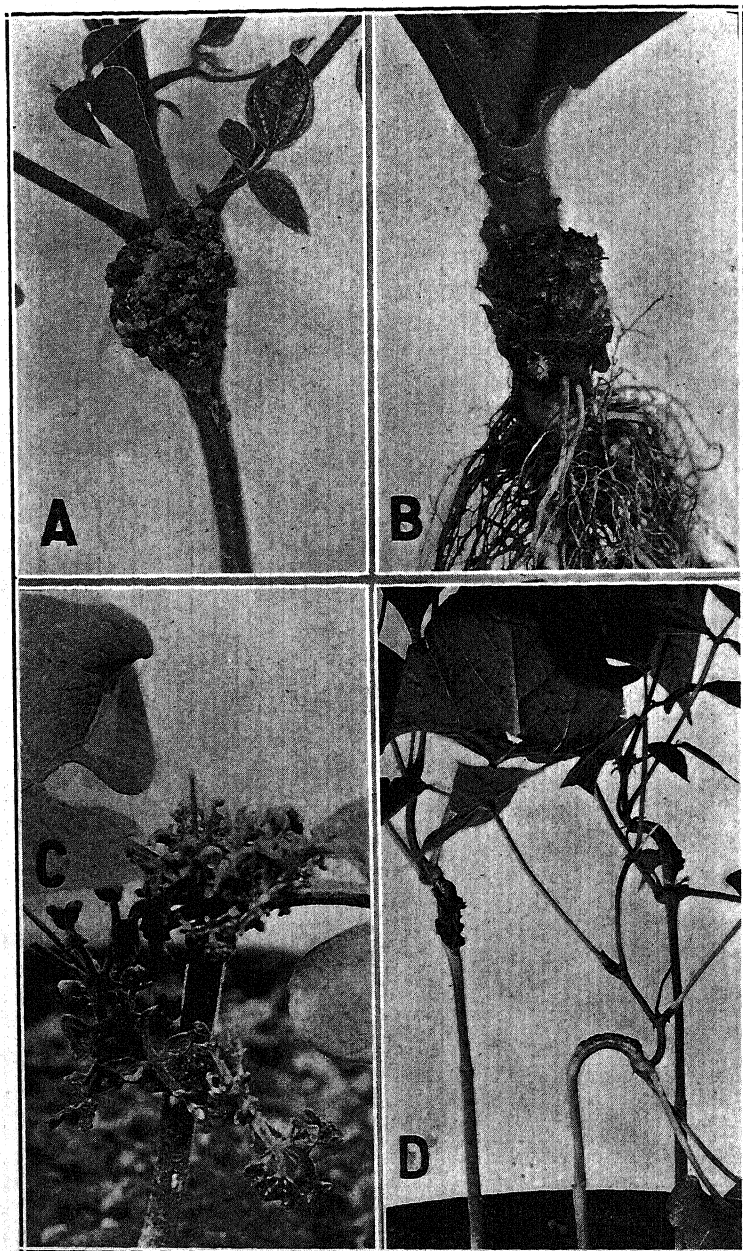


FIG. 2. A. Red Kidney bean seedling wounded at side and smeared with indoleacetic acid February 6, 1936. Photographed April 1, 1936. B. Tobacco seedling wounded and smeared with indoleacetic acid February 21, 1936. Photographed April 14, 1936. C. Very young Red Kidney bean seedling decapitated and smeared with indolepropionic acid February 10, 1936; witches'-broom produced. D. Three Red Kidney bean seedlings smeared on stem with indolepropionic acid January 6, 1936. Photographed February 2, 1936. A-C, a little less than natural size; D, about $\frac{1}{2}$ natural size.



FIG. 3. A. Gall on Red Kidney bean seedling produced by decapitating and smearing with growth substance extracted from sterilized *Bacterium tumefaciens* cultures March 5, 1936. Photographed April 1, 1936. B. Decapitated bean seedling smeared with indoleacetic acid and photographed at the same age as A. C. Another gall on a bean seedling produced by decapitating and smearing with growth substance extracted from sterilized *Bact. tumefaciens* cultures March 5, 1936. Photographed May 22, 1936. A little less than

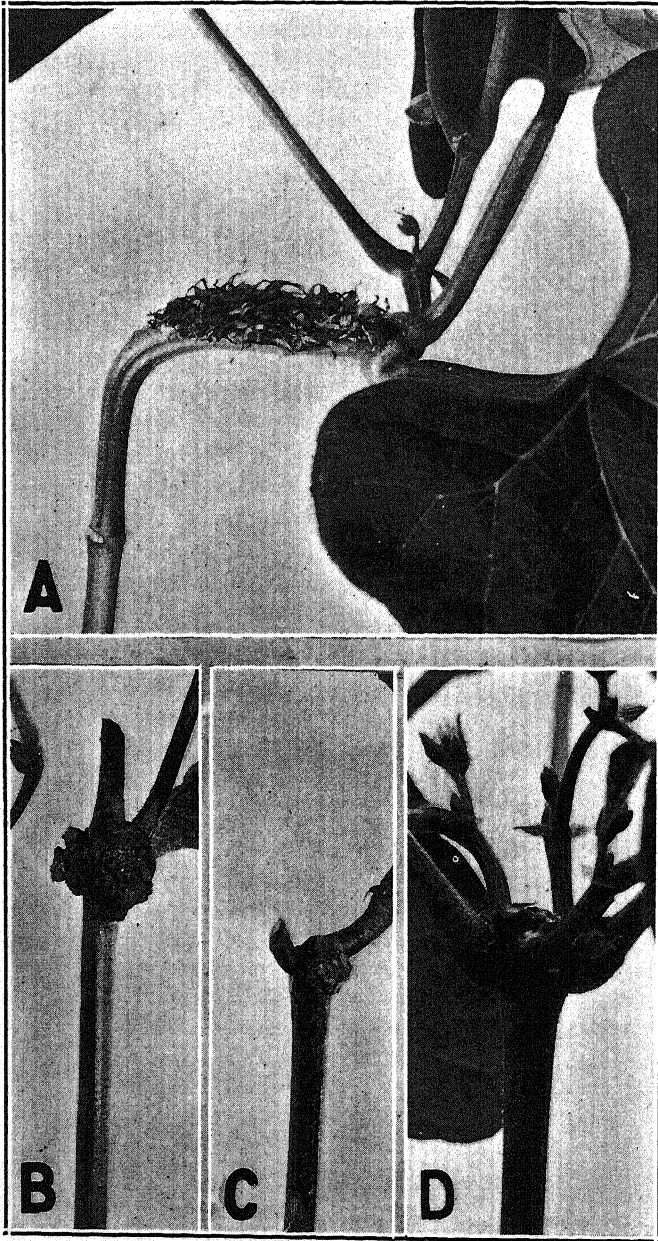


FIG. 4. A. Red Kidney bean seedling smeared with indolepropionic acid January 6, 1936. Photographed February 6, 1936. B. and C. Red Kidney bean seedlings inoculated with *Bacterium tumefaciens*. B. By puncturing. C. By decapitating December 16, 1935. Photographed April 25, 1936. D. Red Kidney bean seedling decapitated and smeared with lanolin only for control. Same age as those in figure 3, A and B. Natural size.

Tomato and bean stems produced bending and root primordia by surface smearing without wounding. (Fig. 2, D and 4, A). Galls with root rudiments and witches'-brooms (Fig. 2, C) were produced on bean plants by decapitating the $\frac{1}{4}$ to $\frac{1}{2}$ in. growth of stem immediately above the first two primary leaves and smearing the wound with indolepropionic acid. Attention might be called here to the fact that witches'-brooms have been produced on tomato and geranium plants by inoculating decapitated stems with *Bacterium tumefaciens* cultures.

Galls produced with a growth substance extracted from Bacterium tumefaciens.—In a theory of gall formation it is not the mere presence of the organism that leads to overgrowths but rather the stimulus of certain products of the organism's metabolism. The preceding work suggested the possibility of producing galls with a growth substance derived from cultures of *Bacterium tumefaciens*.

To this end, the organism was grown in a synthetic medium containing 2 per cent dextrose, 1 per cent peptone (Difco) and a small amount of tryptophane, in addition to the usual inorganic salts, and subsequently extracted with peroxide-free ethyl ether. Upon evaporation of the ether, the residue was taken up, without further purification, in 0.5 g. of pure lanolin.

This lanolin mixture of extracted growth substance was applied to the plants in the manner already described. On bean seedlings (Fig. 3, A and C), and on daisy stems, galls have thus been produced that grow at about the same rate as those produced by indoleacetic acid (Fig. 3, B).

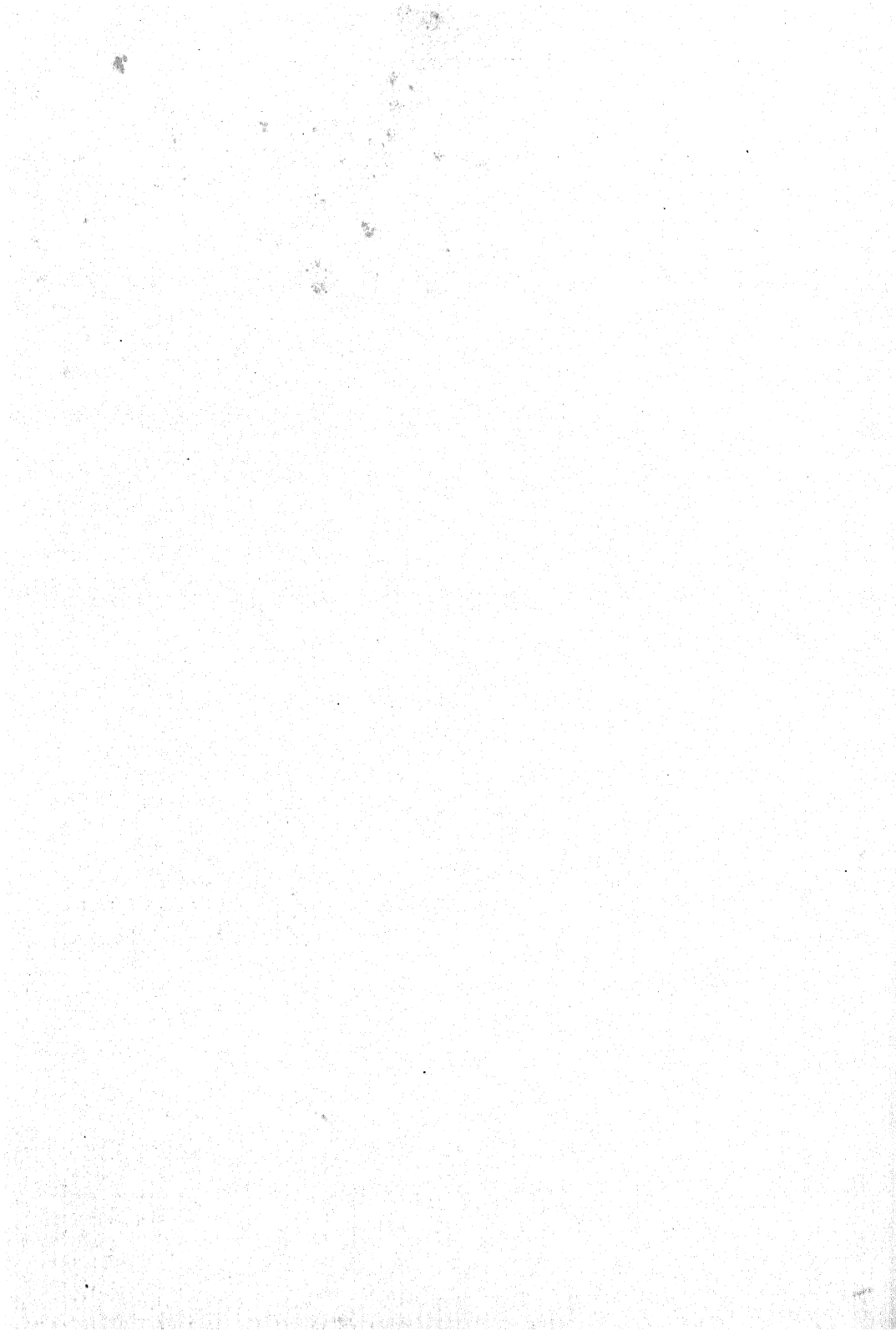
The pure lanolin controls in these experiments have been negative on the various plants (Fig. 4, D). In a few cases nodular growths started on bean plants at the place of lanolin application, but did not continue development beyond a few millimeters.

No definite information relative to the chemical nature of the growth-promoting substance from *Bacterium tumefaciens* or to the best method of its extraction has yet been obtained. Work with these extractions is however, being continued.

NELLIE A. BROWN AND F. E. GARDNER,

BUREAU OF PLANT INDUSTRY,

UNITED STATES DEPARTMENT OF AGRICULTURE.



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SEROLOGICAL TESTS WITH STANLEY'S CRYSTALLINE TOBACCO-MOSAIC PROTEIN¹

K. STARR CHESTER

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The crystalline protein recently reported by Stanley (6) to possess the properties of tobacco-mosaic virus has been made available to the writer for serological tests. The precipitin, complement-fixation, and anaphylaxis techniques have been used in experiments recently completed. It is the purpose of this paper to present the results of these studies.

MATERIALS AND METHODS

Crystalline Tobacco-mosaic Protein. In the tests reported, with the exception of a few that were preliminary, one or another of 5 samples of crystalline tobacco-mosaic protein was employed. The two samples used in most of the experiments were designated as "Crystals I" and "Crystals II," respectively. Both were of protein that had been recrystallized several times by Dr. Stanley. "Crystals I" was not recrystallized during the course of this work, but "Crystals II" was recrystallized twice before it was tested serologically. The details of treatment of the two samples are as follows.

"Crystals I," which was received in November, 1935, was a suspension of protein at a concentration of approximately 1 per cent in ammonium sulphate solution. The suspension was dialyzed against Ringer salt solution for 20 hours in order to remove the ammonium sulphate and to obtain an approximately 1 per cent solution of the protein in Ringer solution. The protein was then precipitated with .5 saturated ammonium sulphate. The precipitate was taken up in a small volume of Ringer salt solution and dialyzed against this solution until the ammonium sulphate had been removed. The resulting solution contained about 2 per cent of protein. The sample designated as "Crystals II" was received in January, 1936. It was in the form of a paste consisting of protein crystals suspended in ammonium sulphate solution. The protein was recrystallized twice under the direction of H. S. Loring. The second crystallization yielded a suspen-

¹ Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in no wise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

sion of crystals that appeared to be free from other materials when examined microscopically. A 2 per cent solution from these crystals was made by suspending an excess of protein in Ringer solution and stirring for 20 hours. This was dialyzed against Ringer solution. The samples designated as "Crystals III, IV, and V" were received in February, 1936. They were submitted as arbitrarily numbered unknowns, and were tested serologically before their identity became known to the writer. After the tests were completed, the information was provided that "Crystals III" was a sample of tobacco-mosaic crystals from mosaic-diseased tobacco, "Crystals IV" was a sample of tobacco-mosaic crystals from mosaic-diseased tomato, and "Crystals V" was similar to "Crystals III," but in a higher state of purification. These 3 samples had been dialyzed against salt solution before they were received, and they were used for serological tests without additional treatment.

Partially-purified Concentrates. Virus in crude frozen juice from tobacco plants having mosaic disease was concentrated by precipitating once or twice with .4 or .5 saturated ammonium sulphate, suspending the precipitate in a small volume of saline solution, and dialyzing against Ringer solution. Three such concentrates were used in these experiments. Several concentrates of the juices of healthy tobacco, mosaic-diseased tomato, and healthy tomato were similarly prepared and used in the tests. The concentrates usually contained about 5 mg. per cc. of protein. Infection tests were used to give assurance that the healthy-plant concentrates contained no virus and that the mosaic-diseased plant concentrates contained abundant virus.

Crude Tobacco Juices. Juices of virus-containing and virus-free tobacco plants were used with no treatment other than freezing, centrifuging, and dialyzing against saline solution.

Other Crude Plant Juices. For purposes of orientation, the anaphylaxis technique was first applied to a variety of viruses and hosts. In these tests crude juices were used. They were prepared in the same manner as the crude tobacco juices. Juices containing the viruses of the following diseases were employed: several strains of tobacco mosaic, tobacco ring spot, peach yellows, peach rosette, little peach, rugose mosaic of potato, mild mosaic of potato, and latent mosaic of potato. The hosts used were potato and tobacco for the potato viruses, peach for the peach viruses, tobacco for tobacco ring-spot virus, and tobacco, petunia, pepper, tomato, and phlox for the tobacco-mosaic viruses.

Precipitin and Complement-fixation Tests. Each of these tests was conducted according to the techniques previously described by the writer (2).

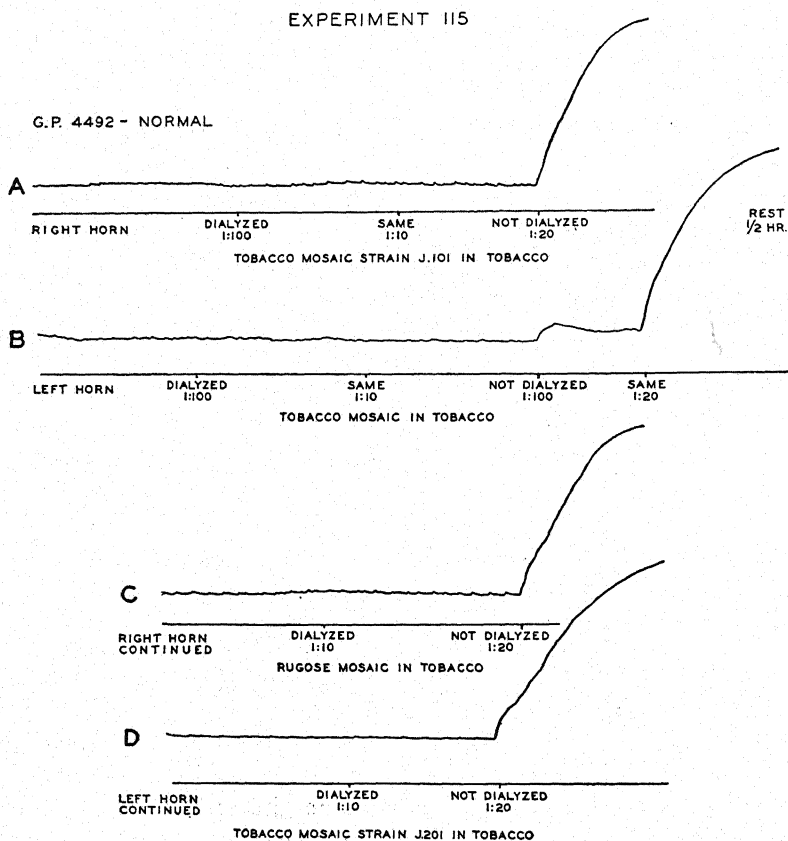
Anaphylaxis Tests. The anaphylaxis test in serology is based on the thesis that an animal injected with a small amount of protein develops,

after about 3 weeks, a high state of sensitivity toward the particular protein used in the sensitizing inoculation. A second inoculation of the same protein after this period causes violent contractions of smooth muscle, frequently resulting in death within a few minutes. The reaction is highly specific. The Schultz-Dale modification of this principle is based on the fact that if a virgin female guinea pig is properly sensitized with a protein, its excised uterus horns, when placed in an isotonic bath, will exhibit contraction when the specific protein used in sensitization is added to the bath. Such contraction may be conveniently recorded on a kymograph drum. The Schultz-Dale modification is somewhat more sensitive than the test in which the animal is reinoculated with protein. It also has the advantage of making possible several tests with the same animal. In the work reported here, the method was used in the manner described by Dale (3), with the exception of certain minor modifications, which will be briefly described.

Since the plant antigens to be studied are normally used in the form of expressed juice, and since the concentration of protein in this juice is relatively low (*e.g.*, healthy-tobacco juice was found to contain only about .2 mg. of protein per cc.), the addition of 1 or 2 cc. of expressed plant juice to the 100-cc. bath usually used may sometimes result in an excessively high dilution of protein. Accordingly, in some of the experiments a bath was used that contained 20 cc. of fluid and permitted the addition of 2 cc. of plant juice, which gave only a 1:10 dilution factor beyond the degree of dilution of the protein in the juice itself. When using such a small bath, it was found desirable to have the plant juices at the same temperature as the bath before adding them, and to have the juices isotonic with the Ringer solution used in the bath. The customary 100-cc. bath was used in comparison with the smaller bath in a number of tests. The comparison showed that the size of the bath did not alter the character of the reactions. The sensitivity of the apparatus was tested by means of experiments in which egg albumin was the antigen used. Reactions were obtained at dilutions consistent with the findings of other workers who have used this technique.

It became apparent early that many plant juices are exceedingly toxic to the excised guinea pig uterus, causing it to contract when the juices are at high dilution. All of the juices tested proved to be so highly toxic that they could not be used without some treatment. Dialysis in a Kunitz-Simms dialyzing apparatus (4) was found to be effective in removing the toxic material from the plant extracts. By dialyzing against Ringer solution it was also possible to replace the unsuitable electrolytes of the juices by the isotonic salt composition of Ringer's solution. Such dialysis usually was continued for 16 to 24 hours, but the toxic material of the potato passed so slowly through the dialyzing membrane that 36-48 hours of dialysis were necessary. The dialyzed extracts could be used freely at a dilution

of 1:10 in the bath without the production of artefact contractions. In the case of anaphylaxis, the muscle tissue shows only one complete reaction toward a given antigen; in the case of the toxic materials in plant juices, the tissues react completely several times in succession, provided they are washed and allowed to relax after each contraction. Thus the two types of reaction may be distinguished.



Photographed by J. A. Carlile

FIG. 1. Effect of dialysis in removing toxic material from tobacco extracts. In each case the dialyzed extract is nontoxic (*i.e.*, induces no muscle contraction), while the corresponding undialyzed extract is highly toxic.

The efficiency of dialysis in eliminating artefact toxic reactions of plant juices is illustrated in figure 1, which gives the kymograph records of the two horns of a normal guinea-pig uterus treated successively with dialyzed and nondialyzed aliquots of virus-containing tobacco juice. Record A shows that the addition of dialyzed tobacco juice at a net concentration of either 1:100 or 1:10 failed to cause contraction or even to interrupt the normal

pulsation of the muscle, while the nondialyzed aliquot of the same juice at 1:20 caused violent contraction. After allowing this muscle to relax, it was similarly tested with dialyzed and nondialyzed aliquots of a second tobacco juice sample, with analogous results (Fig. 1, B). The other horn gave similar results with two other tobacco juice samples (Fig. 1, C and D). In the anaphylactic tests reported in this paper, only extracts were used that failed to stimulate the excised muscle of a normal guinea pig in at least two such control tests. Some of the control tests are shown in the figures.

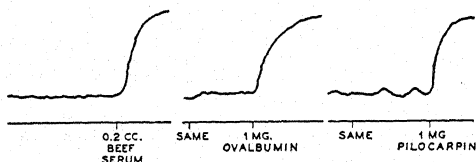
EXPERIMENTAL

1. *Preliminary Anaphylaxis Tests.* The precipitin and complement-fixation tests have been used by a number of workers in the study of plant viruses. By means of these tests, specific reactions for virus materials are obtained. The writer has submitted evidence (1) indicating that it is the virus itself that is the specific antigen involved. The precipitin and complement-fixation reactions with tobacco-mosaic virus are about 25 times as strong as the reactions with the proteins of healthy-tobacco juice. It was learned from preliminary anaphylaxis tests, however, that the healthy-tobacco proteins are highly active anaphylactically. In the purification of tobacco-mosaic virus, the contaminants most difficult to remove are the non-virus proteins of the tobacco plant. This fact suggested the use of the highly sensitive anaphylaxis reaction in the serological study of the crystalline tobacco-mosaic protein; but, before proceeding with this study, orientative tests employing crude and partially-purified virus materials were made.

Previous work with the precipitin and complement-fixation tests had brought evidence that crude plant-virus juice contains two kinds of antigens, namely, proteins normally present in the healthy plant and the specific virus antigen. It is necessary to eliminate the reactions due to healthy-plant proteins, which are present in virus juice, in order to demonstrate reactions due to the virus antigen. For anaphylaxis tests, three ways of eliminating normal host protein reactions suggested themselves, and all were attempted. The three methods were: (a) to permit a sensitized muscle to react fully to healthy-plant juice, and then determine whether there is a residual reaction peculiar to the virus-containing material; (b) to use in sensitizing and testing, respectively, two hosts containing the same virus but so widely separated taxonomically that the only common serological factor would be the virus antigen; and (c) to work with virus materials independently proved to have been freed by chemical means from normal host proteins. The three methods will be discussed in turn.

It is known from the works of numerous investigators that if a guinea pig is sensitized simultaneously to two different proteins, then the excised muscle, when permitted to react completely to an addition of one of these

Guinea pig inoculated with:
0.1cc. beef serum + 1.5gm. ovalbumin
21 days.



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PLANTA 7: 788, FIG. 8.

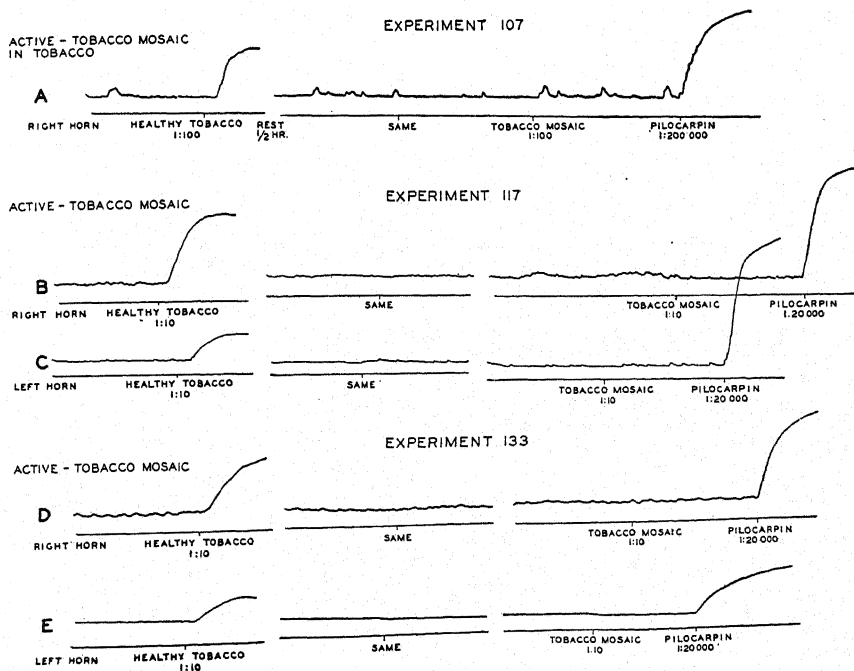
Photographed by J. A. Carlile

FIG. 2. Absorption anaphylaxis reaction with beef serum and ovalbumin. Showing that a guinea pig sensitized with a mixture of these two proteins reacts completely to each in succession.

proteins and subsequently allowed to relax, will show a second complete reaction on the addition of the second protein. Figure 2, which was taken from a record of Moritz (5, Fig. 8, p. 788) illustrates this principle. The guinea pig in question was sensitized with a mixture of beef serum protein and egg albumin. When, after a proper incubation period, the excised muscle was tested against beef serum, it reacted strongly. A second dose of beef serum failed to elicit a reaction, indicating that the muscle had been completely desensitized with respect to that antigen, *i.e.*, its anti-beef-serum anaphylactic antibodies were saturated or exhausted. On the addition of egg albumin, however, a second strong contraction occurred. The first reaction with beef serum did not inhibit the occurrence of a second reaction to a distinct protein. A further dosage of egg albumin failed to cause further reaction, indicating that the antibodies for both proteins were exhausted, although the muscle was still physiologically active, as was shown by the fact that the addition of a small amount of pilocarpin, a nonspecific muscle stimulant, caused a third strong reaction. The principle exemplified in this record was applied to virus materials in the following manner.

Tobacco-mosaic virus was chosen for the first anaphylactic tests. The procedure was to sensitize a guinea pig with 1 or 2 cc. of normal-strength or concentrated juice of mosaic-diseased tobacco plants, and then to test successively against healthy-tobacco juice and virus-containing tobacco juice. The results of 3 experiments of this type are given in figure 3.

The records bring out the following points. Guinea pigs that were sensitized to virus-containing tobacco juice reacted to antigens present in healthy-tobacco juice. This indicates that healthy-tobacco juice and virus-containing tobacco juice contain common antigenic materials. The addition of a second aliquot of healthy-tobacco juice failed to induce contraction,



Photographed by J. A. Carlile

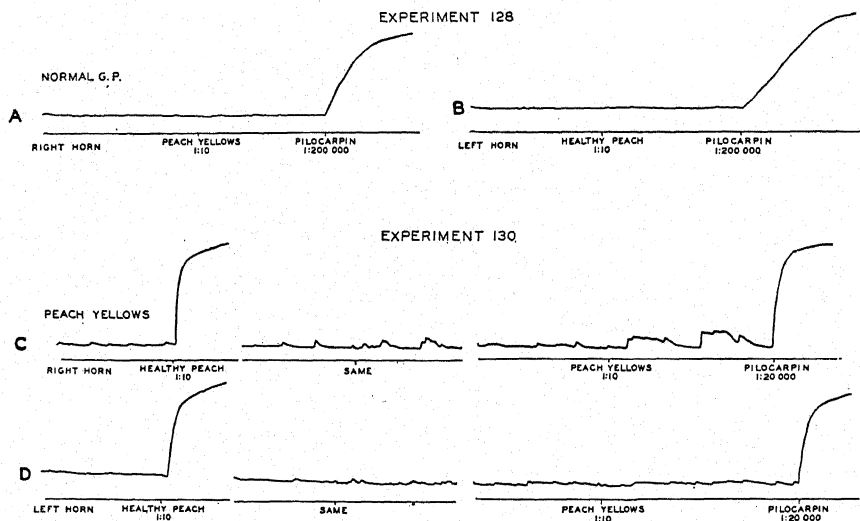
FIG. 3. Absorption anaphylaxis tests with tobacco-mosaic materials. In each case the animal was sensitized to whole extract of tobacco mosaic in tobacco; in each case the muscle reacted to healthy-tobacco protein, but failed later to react to the virus element of the juice, although the muscle was still active as shown by the reaction to the muscle toxin pilocarpin.

showing that the muscles were now saturated with respect to the healthy-tobacco antigens. On adding samples of virus-containing tobacco juice, no further reactions occurred, although in each case the muscle was still in a state of high physiological activity, as was shown by its strong reaction to the muscle stimulant pilocarpin when the latter was at a concentration not greater than 1:20,000 to 1:200,000. Independent tests with normal guinea pigs showed that the healthy-tobacco and diseased-tobacco extracts used contained no detectable nonspecific toxic material at the dilutions employed.

The 3 experiments reported are representative of others performed with tobacco materials and gave comparable results. The materials used for sensitization and the test materials contained appreciable quantities of virus. In some cases the virus in the test material was at 1000 times the concentration necessary to give a precipitin reaction. We know from precipitin tests that the inoculum contained two classes of antigens, virus antigen and healthy-tobacco antigens, yet the animals reacted only toward

the latter. These facts indicate that the virus of tobacco mosaic is not anaphylactogenic when tested by the Schultz-Dale technique.

Similar tests were performed with other plant viruses including the viruses of peach yellows, peach rosette, little peach, rugose mosaic of potato, mild mosaic of potato, latent mosaic of potato, tobacco ring spot, and several strains of tobacco mosaic. All except the peach viruses give good precipitin tests with their specific sera. In all cases the results were similar to those with tobacco mosaic, *i.e.*, the animals sensitized to virus-containing plant juice reacted to the normal plant protein element of the juice but not to the virus element. Figure 4 gives the results of such an experiment with peach



Photographed by J. A. Carille

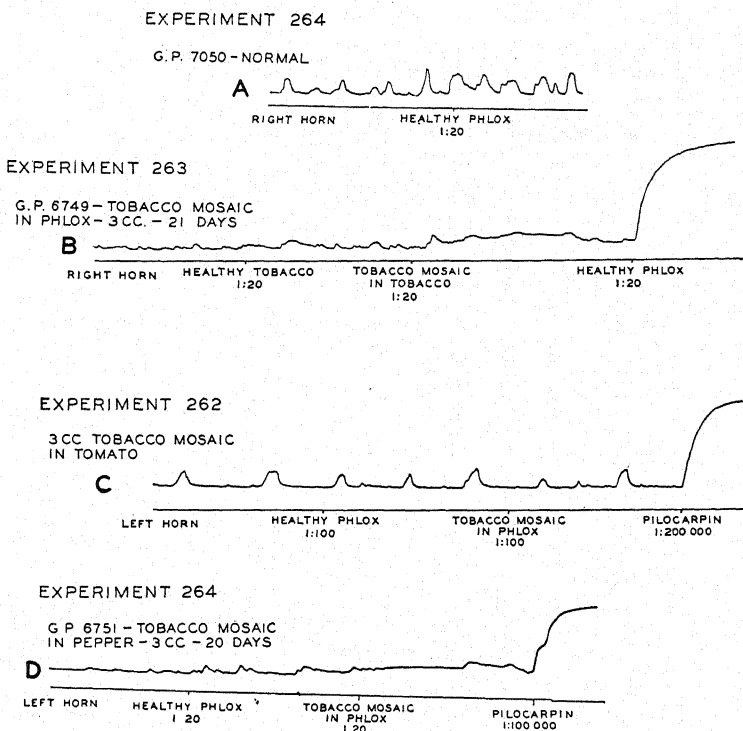
FIG. 4. Absorption anaphylaxis tests with peach-yellows virus materials. Records A and B show that the dialyzed extracts used were nontoxic to normal guinea pig muscles; records C and D as in figure 3, indicating reactivity to the plant-protein element of the extracts but not to the virus element.

yellows. Records A and B show that the two extracts that were intended for use later in the anaphylactic tests did not stimulate the muscles of a noninoculated guinea pig, *i.e.*, were nontoxic at 1:10 dilution, although the normal guinea-pig muscles were highly sensitive to toxic materials, as shown by their reaction to pilocarpin at 1:200,000. Records C and D are of right and left uterus horns, respectively, of a guinea pig sensitized with 2.5 cc. of dialyzed expressed juice from a yellows tree. The two horns were tested in the same manner. Each reacted fully with juice of healthy peach, but after saturation failed to react with yellows juice, although the pilocarpin reactions showed a high degree of sensitivity of the muscles.

The experiments with tobacco-mosaic virus, peach-yellows virus, and the

other viruses mentioned give evidence that none of these viruses is anaphylactogenic. It was felt, however, that further tests were desirable, since it might be that with the plant materials a first reaction against healthy-plant juice in some way inhibits a second reaction against a virus that would otherwise be anaphylactogenic. In view of this possibility, the following type of experimentation was resorted to.

A guinea pig was inoculated with 3 cc. of a potent preparation of the juice of phlox plants infected with tobacco mosaic. After 21 days of incubation the muscles were tested, with results as shown in figure 5.



Photographed by J. A. Carltile

FIG. 5. Anaphylaxis tests of tobacco-mosaic virus excluding reactions due to healthy-host proteins by appropriate choice of hosts. Record A, a control test of toxicity of phlox extract. Record B shows that a guinea pig sensitized with tobacco mosaic in phlox fails to react both with healthy-tobacco extract and with virus-containing tobacco extract. Records C and D indicate that other host plants gave similar results.

The uterine muscle was first tested against an extract of healthy-tobacco juice (Fig. 5, record B). There was no reaction, indicating that phlox and tobacco are too remotely related to contain common antigenic material. Next, the muscle was tested against an extract of virus-containing tobacco juice and again there was no reaction. Since the muscle had not previously

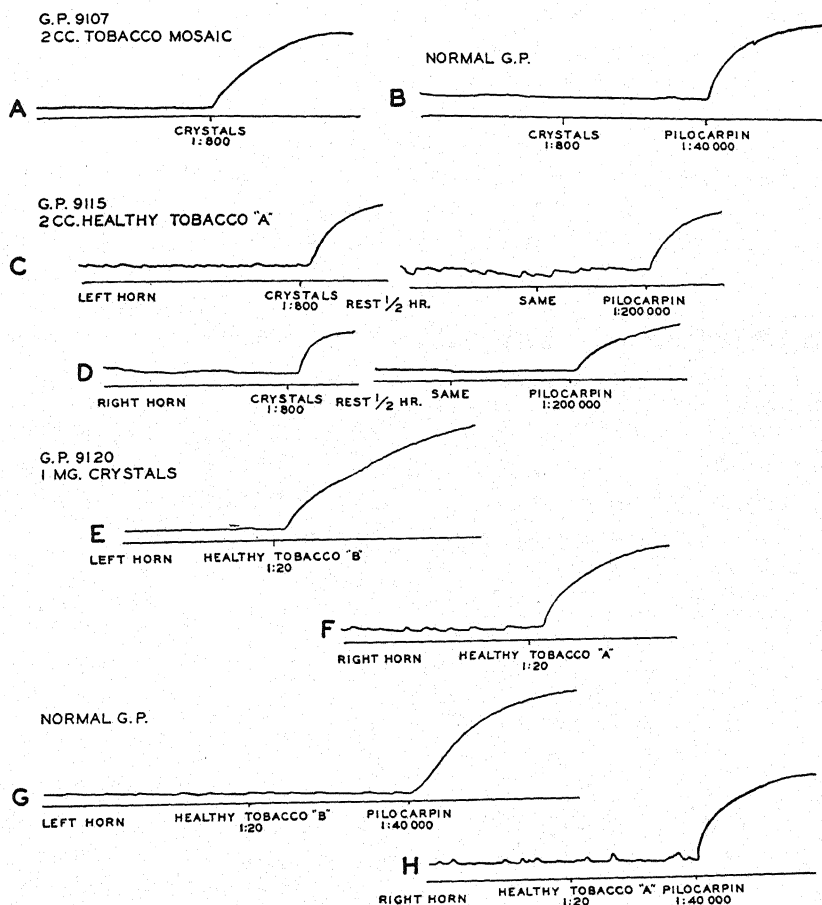
reacted against anything, the failure of virus reaction could not be attributed to inhibition by a previous reaction. Furthermore, there was at least one common antigenic factor in the virus-containing phlox and tobacco juices, namely, the virus. Absence of reaction hence indicates, in conformity with the preceding experiments, that the virus of tobacco mosaic is not anaphylactogenic. That the muscle in question was capable of giving an anaphylactic test is shown by the fact that it later reacted strongly to the juice of healthy phlox, and that this was a true anaphylactic reaction was brought out by the control test (Record A), which showed that the same phlox extract at the same dilution failed to stimulate the muscle of a normal guinea pig.

Records C and D show similar tests using as materials for sensitization tomato and pepper juices containing tobacco-mosaic virus, and as test material phlox juice containing the same virus. In no case was there a reaction that might be ascribed to the virus, although in each case the materials for sensitization and testing contained considerable quantities of infectious matter. These experiments are representative of numerous others that gave analogous results and brought further evidence that tobacco-mosaic virus is not anaphylactogenic.

As already has been stated, precipitin tests afford a highly sensitive index of the presence of tobacco-mosaic virus, but they exhibit a rather low order of sensitivity for the presence of the proteins of the healthy tobacco plant. The data given above indicate that the anaphylactic test is highly sensitive as applied to healthy-tobacco proteins and entirely insensitive toward the virus.

2. *Anaphylaxis Tests of the Crystalline Tobacco-mosaic Protein.* The anaphylaxis test was next applied to the crystalline tobacco-mosaic protein. The records shown in figures 6 and 7 are of tests performed with the sample "Crystals I." The crystalline-protein solution was tested against several normal guinea pigs at a net dilution of 1:800, and in no case caused the normal muscle to contract. Record B in figure 6 illustrates such a test. Two samples of healthy-tobacco juice were used, "A", which was prepared 3 months previous to the tests by precipitating frozen and centrifuged healthy-tobacco juice with 40 per cent saturated ammonium sulphate and by taking up the precipitate in 1/35 the original volume of diluent, and "B" which was prepared 3 months later in a similar manner but using 50 per cent ammonium sulphate. These two healthy-tobacco concentrates were non-stimulatory to normal guinea-pig uteri, as is shown by the records G and H in figure 6. Three such control tests were performed for each extract. The noncrystalline tobacco-mosaic sample used in the experiment was prepared similarly to the healthy-tobacco juice, but by using two successive precipitations with 40 per cent ammonium sulphate. All of the extracts were

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Photographed by J. A. Carlile

FIG. 6. Anaphylaxis tests with crystalline tobacco-mosaic protein. Guinea pig 9107, sensitized with whole tobacco-mosaic tobacco extract, reacted with the crystals (Record A) which were shown to be primarily nontoxic (Record B). This reaction must have been due to healthy-tobacco protein, and not to virus, since healthy-tobacco extract sensitized to the crystals (Records C, D), and since 1 mg. of the crystals sensitized to healthy-tobacco protein (Records E, F). Records G and H are toxicity control tests of the tobacco extracts used.

dialyzed against Ringer solution before testing, in order to remove any toxic substances that might have been present.

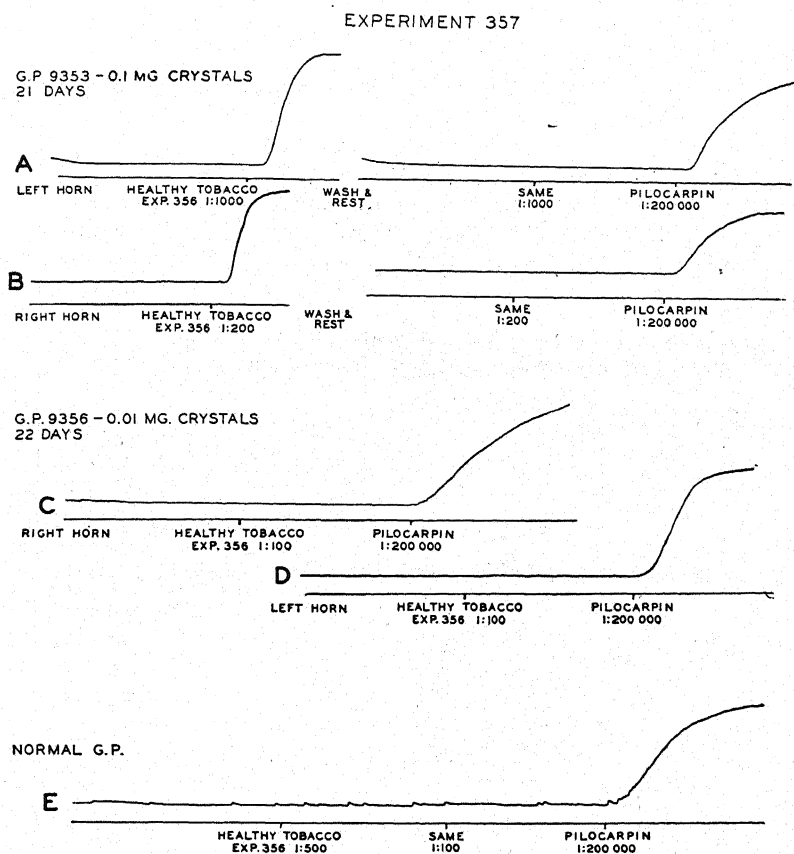
It was first determined that a guinea pig sensitized with crude juice of a mosaic tobacco plant reacted strongly with a 1:800 solution of the crystalline protein (Fig. 6, record A. The reaction of the right horn is shown; the left horn gave a similar reaction.) Since the virus is not anaphy-

lactic, this reaction is not believed to have resulted from virus in the crystalline protein, but from healthy-tobacco protein associated with the crystals. Subsequent tests showed that the muscles of animals inoculated with mosaic-diseased tobacco juice react with healthy-tobacco proteins, and that after they have been desensitized with healthy-tobacco proteins, they show no residual reaction against the crystals.

Guinea pig 9115 received an inoculation of healthy-tobacco protein, containing no virus. Its muscles reacted with the crystalline material. This is considered to be a true anaphylactic test, since the reaction could not be repeated with the same muscle and, since the crystalline protein was non-stimulatory to normal guinea-pig muscles. Each of two guinea pigs was inoculated with 10 mg. of the crystalline material; a third with 3 mg., and a fourth with 1 mg. Each of the 8 muscles reacted when either of the healthy-tobacco extracts was added to the bath. The reactions of the pig receiving 1 mg. of the crystalline protein are illustrated in figure 6 (Records E and F). Since the reactions were strong, it seemed probable that still smaller amounts of "Crystals I" would sensitize pigs to healthy-tobacco protein. Accordingly, a second experiment of the same type was performed, this time using guinea pigs sensitized with "Crystals I" in dosages of 1 mg. (1 pig), .3 mg. (2 pigs), .1 mg. (2 pigs), .03 mg. (2 pigs), and .01 mg. (2 pigs). The pigs receiving 1 mg., .3 mg., and .1 mg. all reacted strongly with the healthy-tobacco concentrate, even when the latter was diluted 1:1000 (.005 mg. protein per cc.). Some of these tests are shown in figure 7 (Records A and B). Of the two pigs receiving .03 mg. of crystalline protein, one was negative toward healthy-tobacco concentrate, while the other reacted weakly with this juice. Both pigs receiving .01 mg. were negative when tested against the healthy-tobacco concentrate, although highly sensitive to the muscle stimulant, pilocarpin (Records C and D, Fig. 7). The healthy-tobacco concentrate used was not stimulatory to 3 normal guinea pigs. A record from one of these control animals is shown in figure 7 (Record E). Infection tests on *Nicotiana glutinosa* L. showed that the healthy-tobacco concentrate contained no detectable virus. The minimal sensitizing dose of this sample of crystalline protein for reaction with healthy-tobacco protein thus appeared to be in the vicinity of .03 mg. for 200 gm. guinea pigs.

Further anaphylactic tests were performed with the samples of "Crystals II, III, IV, and V." These samples seemed to show a higher state of purity than "Crystals I," as the minimal sensitizing and desensitizing doses required were somewhat greater. The results of all of the tests are grouped in table 1.

Certain of the data given in table 1 may be summarized as follows. From a total of 97 tests, it was evident that there was regular interreaction between crystals from tobacco or tomato and healthy tobacco or tomato. The



Photographed by J. A. Carlile

FIG. 7. Anaphylaxis tests with crystalline tobacco-mosaic protein. Records A and B show that a guinea pig, sensitized with .1 mg. of crystalline protein, reacted strongly with healthy-tobacco concentrate at 1:1000 (.005 mg. protein per cc.). The reaction was a true anaphylactic test, since the tobacco concentrate at 1:500 or 1:100 was nontoxic to a normal guinea pig (Record E), and since a second administration of the tobacco concentrate to a sensitized pig failed to elicit a reaction. A second guinea pig (Records C, D) was not sensitized by .01 mg. of the crystalline protein. The minimum sensitizing dose for this sample of crystals was apparently in the vicinity of .03 mg.

evidence from 43 tests showed a consistent cross reaction between juices of tobacco and juices of tomato. When tobacco-mosaic virus in one host was used for sensitization and the same virus in a second host for desensitization, there was no reaction attributable to the virus, according to 11 tests, exclusive of the tobacco-tomato reactions. Thirty-five negative tests in which animals were sensitized with virus in a given host, desensitized with healthy juice of the same host, and then tested against virus-containing juice, gave further evidence that the virus is not anaphylactic when tested by the Schultz-Dale technique.

TABLE 1.—*Summary of anaphylaxis tests of tobacco-mosaic crystals, including control tests of healthy host juices*

Sensitization	Results of tests	No. of tests
Crystalline tobacco-mosaic protein from tobacco. ("Crystals I, II, V")	With crystalline tobacco-mosaic protein from tobacco, positive at high concentrations, .1 mg. or more usually required to sensitize. Regularly positive with healthy tobacco and healthy tomato at high concentrations.	96
Crystalline tobacco-mosaic protein from tomato. ("Crystals IV")	Positive at high concentrations with healthy tobacco, healthy tomato, crystals from tobacco, and crystals from tomato.	12
Healthy tobacco	Strongly positive even at high dilutions with healthy tobacco and healthy tomato. Positive at high concentrations with crystals from tobacco.	68
Healthy tomato	Strongly positive with healthy tobacco and healthy tomato, weakly positive with crystals from tobacco.	12
Crude juice of tobacco mosaic in tobacco	Strongly positive with tobacco mosaic in tobacco, healthy tobacco, healthy tomato, crystals from tobacco, and latent potato mosaic in tobacco. Negative with virus-containing tobacco, tomato, or crystals from tobacco after desensitization with healthy tobacco. Negative with tobacco mosaic in phlox.	92
1 masked and 4 yellow strains of tobacco mosaic in tobacco	Positive with healthy tobacco and with crystals from tobacco. Negative with virus-containing juices after desensitization with healthy tobacco.	19
Crude juice of tobacco mosaic in tomato	Strongly positive with healthy tobacco and virus-containing tobacco. Negative with healthy and virus-containing phlox. Negative with virus-containing tobacco or tomato after desensitization with healthy tomato.	32
Crude juice of tobacco mosaic in phlox	Negative with healthy and virus-containing tobacco, strongly positive with healthy phlox.	13
Crude juice of tobacco mosaic in pepper	Negative with virus-containing tobacco and phlox.	4

From the experiments reported in table 1, the following conclusions are drawn. Crystals of tobacco-mosaic protein from tobacco and healthy-tobacco juices contain common antigenic material. This applies to materials recently crystallized, as well as to earlier preparations of crystals. The reaction between crystals and healthy-tobacco protein is not regarded as due to a cross reaction between virus and some protein of the healthy host plant that may serve as a precursor of the virus, because the experiments indicate that virus is not anaphylactic when tested by the Schultz-Dale technique. It is, therefore, concluded that there are present in the solutions of crystals proteins that are found in the healthy tobacco plant, and, accordingly, are looked upon as contaminations of the virus in the crystals. The anaphylaxis results thus far available do not warrant conclusions as to the amount of this contamination.

The positive cross reactions between crystals from tobacco and crystals from tomato are not considered to indicate that the virus in these two preparations is anaphylactic and identical, because the experiments show that healthy tobacco and tomato contain common anaphylactogenic material, which has thus far not been completely removed in the process of crystallization. Absorption tests were used to differentiate healthy tobacco and healthy tomato, but as a rule the tests indicate that a large proportion of the protein of these two species is anaphylactically alike.

A comparison of the precipitin and anaphylactic tests with tobacco-mosaic virus leads to the question of why the virus, which is strongly reactive by the precipitin test, fails to show anaphylactic activity even when at a concentration 1000 times as great as the least concentration giving a precipitin test. Although this can not be answered at the present time, it is possible that the large molecular weight of the virus (estimated by Stanley (6) to be of the order of "a few millions") prevents the free diffusion of the antigen into the isolated guinea pig muscle. Protected as the muscle is by several relatively impervious coatings, it is to be expected that the larger the molecule of antigen the greater will be the difficulty of penetration to the probable locale of the antibodies, in the reticulo-endothelial system of the muscle tissue. Hence, the larger the molecule the less activity would be shown anaphylactically. On the other hand, a large molecule would probably be more easily precipitated than a small molecule of protein, other factors remaining constant. The relatively poor solubility of the crystalline protein might likewise be expected to favor precipitation and to interfere with anaphylactic action.

It is evident from the anaphylactic tests that there are present in the mosaic-diseased plant no anaphylactically active antigens other than those allied or identical to the healthy-host proteins, because an animal sensitized with virus-containing plant juice can be completely desensitized with the

juice of the healthy suscept. The possibility often has been considered in the literature that the plant-virus precipitin reaction is not due to the virus but to some serologically active specific by-product of virus activity. The anaphylaxis tests fail to give evidence of the presence of any such specific virus by-product. The experiments do not exclude the possibility that there may be specific, non-anaphylactic products of virus metabolism.

3. *Complement-fixation Tests.* Complement-fixation tests were performed for two purposes, to determine the presence and amount of virus in the crystalline material, and to ascertain whether or not the crystals contained antigenic material present in virus-free tobacco plants that could be demonstrated by this technique. Table 2 gives in condensed form the results of 3 experiments of this type.

TABLE 2.—*Complement-fixation tests^a with crystalline tobacco-mosaic protein*

Antigen	Immune serum	Reaction
1. Crystals	Anti-crystals	Complete fixation to 1: 10,000,000 ^b
2. Crude tobacco-mosaic juice	Anti-crystals	Complete fixation to 1: 2,500,000 ^b
3. Crystals	Anti-healthy-tobacco	Complete fixation to 1: 660, not above ^b
4. Crude healthy-tobacco juice	Anti-healthy-tobacco	Complete fixation at 1:100, nearly so at 1: 300 ^c

^a *System:* .3 cc. immune serum at 1: 10 or 1: 15 + .2 cc. fresh guinea pig complement at 1: 10 or 1: 15 + 1.0 cc. antigen dilution + 1 hr. at 37° C. + .5 cc. sensitized sheep cells (= .25 cc. 1: 500 or 1: 750 inactive anti sheep amboceptor + .25 cc. 4 per cent washed sheep cells + 1 hr. in cold) + 1 hr. at 37° C.

^b Dilution of antigen in terms of total protein.

^c Dilution of antigen solution with original concentration of juice taken as 1: 1.

The writer has previously shown that tobacco-mosaic juices interact by the complement-fixation method at high dilutions, and it is believed that this reaction is due to the virus itself (1). In table 2, reactions 1 and 2 are considered to be due in greatest part to the interreaction of the virus element of the test materials. Reaction 3, however, shows that the crystals also react with healthy-tobacco antiserum, although not at high dilutions. When healthy-tobacco juices are used as antigens with healthy-tobacco antiserum (reaction 4), reaction occurs but only at relatively high concentrations. It is not felt that one should draw conclusions from these data as to the absolute concentration of healthy-tobacco antigen in the crystals, since the quantitative features of the tests are relatively poorly known. The complement-fixation tests are in agreement with the anaphylactic tests in show-

ing the presence in the crystalline protein solutions of non-virus proteins of the healthy tobacco plant. They show in addition that the crystals contain considerable amounts of virus antigen.

4. *Precipitin Tests.* As has been indicated in the preceding pages, the expressed juice of a mosaic-diseased tobacco plant contains two groups of antigens, (a) a complex of proteins such as are present in healthy tobacco plants, and (b) the virus antigen. Precipitin tests have given no indication as yet of a third group, namely, proteins peculiar to the disease but distinct from the virus. It is known from the work of several investigators that the virus antigen of tobacco mosaic is highly precipitinogenic. On the other hand, the healthy-tobacco protein complex has a low order of antigenicity as shown by the precipitin tests. The precipitin test is entirely suitable for detecting virus in tobacco juices, but not well-adapted for detecting healthy-tobacco proteins. Crude virus juice may be diluted 1:250 before the virus precipitin reaction disappears, while the healthy-tobacco protein reaction disappears at 1:10 dilution of the same juice. However, precipitin tests were conducted in order to obtain evidence on the concentration of virus in the crystals, and if possible to shed some further light on the problem of the interreaction of crystals and healthy-tobacco protein. Table 3 gives a summary of the results of one of 10 experiments on this subject.

The data indicate that the crystals contained large amounts of virus antigen. The solutions of crystalline protein above 1:660 dilution failed to react with healthy-tobacco protein immune sera. Below this dilution the solutions of crystalline protein were so opalescent that an end-point reaction could not have been detected had there been one. Crude healthy-tobacco juice, however, reacted with its specific immune serum at no higher dilution than 1:10. It is believed that the failure of the crystals to react with anti-healthy-tobacco sera is due to the low sensitivity of the precipitin test for healthy-tobacco protein and to the opalescence of the more concentrated crystalline-protein solutions that makes it difficult or impossible to detect weak precipitin reactions. Experiments have been conducted that were designed to bring out the presence of a precipitin-inhibiting substance in the crystal solutions, but no evidence of such a substance was obtained. In addition to the tests summarized in table 3, precipitin tests for the purpose of detecting virus also were performed with "Crystals III, IV, and V." Solutions of each of these crystalline preparations reacted strongly with absorbed serum prepared against tobacco-mosaic-virus-containing tobacco juice.

During the course of the anaphylactic investigations, blood was drawn from each of the anaphylactic guinea pigs used, and the sera of these bloods were stored. At the conclusion of this work it was felt desirable to test

TABLE 3.—*Precipitin tests of crystalline tobacco-mosaic protein*

Antigen	Serum		Serum contained anti-bodies for	Reaction, definitely positive to antigen dilution ^b
	Type	Dilution		
Crystals I	Anti-tobacco-mosaic, absorbed ^a	1:3	Virus only	1:66,000
"	"	1:1	"	1:200,000
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	None above 1:660; see text
"	"	1:1	"	"
"	Normal, absorbed	1:3	None
"	"	1:1	"
"	Normal, not absorbed	1:3	"
"	"	1:1	"
Crystals II	(All tests as with Crystals I, all giving results identical to those with Crystals I, except virus reactions, which were somewhat weaker.)			
Crude tobacco-mosaic juice	Anti-tobacco-mosaic, absorbed	1:3	Virus only	1:25,000
"	"	1:1	"	1:75,000
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	1:10
"	"	1:1	"	1:10
(Normal serum tests as with Crystals I, all negative.)				
Crude healthy tobacco juice	Anti-tobacco-mosaic, absorbed	1:3	Virus only	None
"	"	1:1	"	"
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	1:3
"	"	1:1	"	1:10
(Normal serum tests as with Crystals I, all negative.)				
0 × concentrated healthy tobacco juice	Anti-tobacco-mosaic, absorbed	1:3	Virus only	None
"	"	1:1	"	"
"	Anti-healthy-tobacco, not absorbed	1:3	Tobacco protein only	1:100
"	"	1:1	"	1:100
(Normal serum tests as with Crystals I, all negative.)				

^a *Absorption*: 1 part serum added to 2 parts crude healthy-tobacco juice, incubated 2 hrs. at 37° C. + 16 rs. at 5° C., then centrifuged and precipitate rejected.

^b Dilution of crystals and crude virus juice expressed in terms of total protein, dilutions of healthy-tobacco juices with original concentration of juice taken as 1:1.

these sera for precipitins, since preliminary experiments had shown that the serum of an anaphylactic guinea pig frequently contains precipitins for the antigen used in sensitization. The sera from 105 of the anaphylactic and normal guinea pigs used in the preceding experiments were tested against the juices of healthy tobacco, mosaic-diseased tobacco, and tobacco diseased with latent mosaic of potato. These tests brought out the following points. Guinea pigs that were sensitized with healthy tobacco and showed strong anaphylactic reactions with healthy tobacco, yielded sera that failed to give evidence of precipitins for healthy-tobacco proteins. As has been pointed out, healthy tobacco proteins are also poor precipitinogens in the rabbit. The sera of animals sensitized with tobacco-mosaic crystals or with the juices of mosaic-diseased tobacco also failed to react with healthy-tobacco juice. The sera of guinea pigs sensitized to either crude mosaic-virus-containing tobacco juice or tobacco-mosaic crystals reacted with virus-containing tobacco juice, provided the sensitizing dose was .3 mg. of protein or more. It frequently happens that individual animals fail to become anaphylactically sensitized when inoculated with doses which sensitize other pigs. In a number of cases guinea pigs were sensitized with 1.0 mg. of crystals and failed in the anaphylactic tests to show sensitivity toward crystals. When sera from such anaphylactically inactive pigs were tested for virus precipitins, they were found to give precipitin reactions as strong as those given by the sera of sensitized pigs. These facts indicate that the anaphylactic test is a much more sensitive test for healthy-tobacco proteins than the precipitin test, and that production of precipitins in sensitized guinea pigs develops independently of the production of an anaphylactic state. In the same animal, tobacco-mosaic virus is a highly active precipitinogen and inactive anaphylactically, while healthy-tobacco proteins are inactive by the precipitin method but highly active anaphylactically. This implies that the mechanisms for the production of the two reactions in the same animal are quite different, even though the same antibodies may be concerned.

SUMMARY

1. The Schultz-Dale method was applied to materials containing several plant viruses, including tobacco-mosaic virus. It was found that the viruses tested gave no anaphylactic reactions. This was shown both by absorbing the muscles with healthy-plant juices prior to testing for virus reaction, and by using as virus hosts for sensitization and testing, respectively, two species of plants so remotely related that the virus was the only common serological element. Healthy-plant proteins, on the contrary, were highly anaphylactogenic. The proteins of healthy tobacco and healthy tomato were very similar serologically.

2. The uteri of animals sensitized with healthy-plant proteins reacted to solutions of crystalline tobacco-mosaic virus protein, and the uteri of animals sensitized with the crystalline protein reacted to dilute extracts of healthy-tobacco proteins. Complement-fixation tests confirmed the anaphylactic tests in showing cross reactivity between the crystalline protein and healthy-plant protein. The evidence indicates that this cross reactivity is due not to a serological affinity between virus and healthy-tobacco protein, but to the presence in the crystalline material of a contaminating protein serologically allied or identical to protein of the healthy tobacco plant.

3. Precipitin and complement-fixation experiments revealed the presence in the crystalline materials of considerable quantities of virus.

4. Precipitin tests of the sera from sensitized guinea pigs showed that in a given animal tobacco-mosaic virus may be a highly active precipitinogen but inactive anaphylactically, while healthy-tobacco proteins in the same animal may be comparatively inert in producing precipitins but highly active in stimulating anaphylaxis. This implies that the mechanisms of the two reactions are different, although the same antibodies may be concerned in both. It is possible that the molecular size or solubility of the respective antigens underlies this difference in antigenic manifestation.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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RELATIONSHIP OF CLIMATOLOGICAL CONDITIONS TO THE TOBACCO DOWNY MILDEW

LAWRENCE F. DIXON, RUTH A. MCLEAN, AND FREDERICK A. WOLF¹

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Weather conditions have long been known to influence the prevalence and destructiveness of certain plant diseases, notably those caused by the rusts or by the downy mildews. Our previously recorded conclusions,² as to the effect of weather conditions upon the course of the downy mildew disease of tobacco, were based upon observations made during the season of 1933. Subsequent observations, made during the two following years, support the general conclusion, previously drawn, that the progress of this disease is closely circumscribed by meteorological conditions. The correctness of certain other conclusions, especially those dealing with the influence of cloudy, rainy weather in promoting sporulation, also are substantiated by our further experimentation. It appears, however, that such weather may not be favorable, as was once believed, for the wide dissemination of sporangia. Certain other conclusions also must be modified in the light of the additional observations. The results of 3 years' experience have shown that, even though the type of weather may differ each spring, the course of the downy-mildew disease is similar in certain features each year and appears to be predictable. It is the present purpose, therefore, to compare and contrast the pertinent observations and data and to correlate the course of the disease with climatological conditions. Such a treatment should contribute to a better understanding not only of this disease but of other diseases whose progress is conditioned by meteorological factors.

The sources of information from which these correlations have been made are (1) records obtained at field stations, (2) surveys made in representative areas of the State, and (3) climatological data compiled by the Weather Bureau of the United States Department of Agriculture.

Two field stations were established each season at which to make daily observations and to record the progress of the downy-mildew disease. The one, located in eastern North Carolina, was abandoned when the disease became generally prevalent in that locality, and the other was then established in the Piedmont section. A continuous record of temperature and humidity was secured at each station by means of a Friez hygrothermograph placed in a seed bed. In addition, a continuous record was made, in 1935,

¹ Thanks are extended to Dr. P. M. Gross for his criticisms in preparation of this paper.

² Wolf, F. A., L. F. Dixon, R. McLean, and F. R. Darkis. Downy Mildew of Tobacco. *Phytopath.* 24: 337-363. 1934.

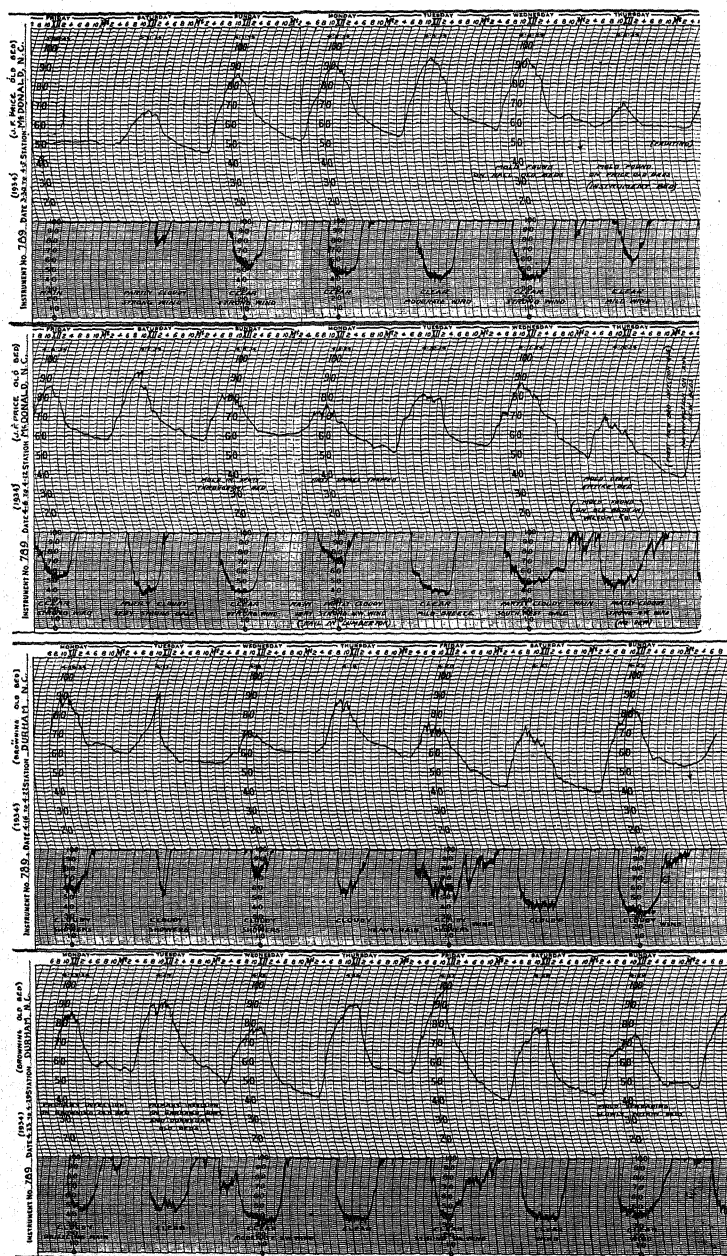


FIG. 1. Upper half, continuous record of the temperature of the air and of the humidity within a seed bed at McDonald, N. C. for the period Mar. 30-Apr. 12, 1934. Lower half, similar record, at Durham, N. C. for the period Apr. 16-Apr. 29, 1934.

of the air temperature just outside the seed bed and of the soil temperature within the seed bed at a depth of approximately $\frac{1}{2}$ in. Records of the air temperatures outside the selected seed beds are not presented. The records, shown in figures 1-5, are, therefore, those of conditions in specific seed beds, but the daily observations took into account the course of the disease in a considerable number of seed beds within the radius of a mile or more of the one containing the self-recording instruments. Our exact knowledge of climatological phenomena and the progress of the disease are, therefore, based upon conditions obtaining in small areas. The data are so interpreted, however, as to make the results widely applicable by supplementing these data with information gained both from surveys of conditions in widely separated areas and from data taken from the reports of the Weather Bureau.

GENERAL STATEMENT OF WEATHER CONDITIONS AND OF THE COURSE OF
THE DOWNY MILDEW DISEASE FOR THE YEARS 1931-1935

Weather conditions existing prior to and during the outbreak of downy mildew of tobacco influence the time when primary outbreaks occur, as well as the rate of the subsequent spread and destructiveness of the disease. In order to provide a background for the better understanding of the meteorological data presented in figures 1-5, consideration will be given to the general weather conditions, the progress of vegetation, and the behavior of the disease for the period 1931 to 1935, inclusive.

In 1931. Normal temperatures prevailed throughout January. Since the rainfall was 1.7 inches below normal, and the precipitation previously had been deficient, conditions were favorable for plowing in preparation for spring.

February was generally mild, with temperatures that averaged 1.5° F. above normal. The rainfall was 2.3 inches below normal, as a result of which the stream flow continued low. Small grain and truck crops were adversely affected by lack of moisture.

March was uniformly cold, with a mean temperature 5.5° F. below normal; the coldest March weather within 15 years. Rainfall was well distributed but only about 50 per cent of normal. Much field work was done during the month. Low temperatures retarded plant growth. During the last week of the month downy mildew of tobacco appeared for the first time in seed beds in Louisiana.

The mean temperature in April averaged 1.7° F. below normal. There were several mild periods but no markedly warm spells. Rainfall was 0.8 inch above normal, and was well-distributed, causing overflow of the lower levels of rivers. Crops made good to excellent growth. Downy mildew of tobacco appeared in Georgia during the first week of April, and the first occurrence in North Carolina was reported on April 27. It was then esti-

mated that the disease had been present since the week of April 19-25. Attention also may be directed to its occurrence on large plants in many fields in 11 counties in Georgia, during the last week of April and in early May.

Weather during May generally cold, with average temperature 1.9° F. below normal. Rainfall well-distributed throughout the State and 0.7 inch above normal, resulting in flood stages in many streams. Cold weather retarded growth of all crops. By May 8, downy mildew present in a few seed beds in nearly all counties in southeastern North Carolina. A week later it was reported present in fully half of the beds in this area.

Because of lateness of the outbreak, losses from downy mildew in North Carolina were inconsequential in 1931, the first year of its recurrence in the southeastern United States.

In 1932. January, similar to December, unusually warm. Monthly mean temperature 9.1° F. above normal and almost as high as highest recorded January (1890) temperature. No cold periods occurred during entire month. For 6 days (Jan. 13 to 18) maximum temperature averaged 73° F. and minimum, 54° F., at Lumberton, warm enough to initiate a primary outbreak of downy mildew. Such an assumption is warranted by the observed premature swelling of peach flower buds and the flowering of hardy shrubs and roses. The disease was noted in Georgia on Jan. 21. Rainfall 1 inch above normal; soil too wet for plowing.

February continued exceptionally mild. Monthly mean temperature 6.9° F. above normal, almost the highest mean for that month since 1890. Weather favorable for primary infections occurred at Lumberton from Feb. 10 to 14, when maximum temperature averaged 70° F. and minimum 50° F. The disease was actually reported in this area Feb. 23. It is quite certain that it had progressed to secondary stages before being discovered. An outbreak of downy mildew was reported in Georgia on Feb. 8. Rainfall averaged 1 inch below normal. Eastern counties received 50 per cent of normal amount. Small grain and truck made favorable growth and many shrubs, bulbs, fruit trees, and roses bloomed prematurely.

The mild weather of the 3 preceding months continued until March 6, when a severe storm occurred, followed by a severe freeze that extended to the coast during the following 10 days. Average precipitation 0.4 inch above normal. Strong winds general on March 22, 27, and 28, which were conducive to dissemination of downy-mildew sporangia. The cold wave destroyed the early strawberry crop and damaged truck, grain, and fruits. Numbers of tobacco seed beds were destroyed or severely damaged. The widespread use of sprays for downy-mildew control also adversely affected tobacco seedlings. Monthly mean temperature 3.4° F. below normal.

April temperatures about normal and average rainfall 1.2 inches below

normal. The State as a whole received only two-thirds normal rainfall. Conditions favored farm work, but cool weather and insufficient moisture delayed planting 7 to 10 days. Tobacco plants were extremely scarce, and many seed beds were in very poor condition as a result of unfavorable weather, damage by downy mildew, and widespread and injudicious spraying. This resulted in approximately 50 per cent reduction of the 1932 tobacco crop.

May rather cold, averaging 1.0° F. below normal; rainfall averaged 0.4 inch below normal. River stages, below normal. Weather conditions poor to fair for farm work and crop growth. Tobacco varied from poor to fair. Many plants lost in beds and fields because of downy mildew and spray injury. Much replanting, crop made a very late start. During the last week of May downy mildew was noted in the field on plants that were about 2 feet high.

The 1932 tobacco crop was the poorest in quality and quantity in many years. This was attributed to adverse weather conditions, damage by downy mildew and by sprays, insufficient seed-bed areas, and lack of knowledge regarding the handling of seed beds and of transplanting when downy mildew was present.

In 1933. January was abnormally warm; temperature, considerably above normal during about one-half of the month, averaged from $+4^{\circ}$ to $+10^{\circ}$ F.; caused abnormal swelling of fruit tree buds and blooming of early shrubs. Precipitation was 0.8 inch below normal; soil, in general, too wet for plowing.

February was characterized by much cloudiness, frequent rains, and moderate temperatures. Two cold waves occurred, one of 2 days' and the other of 4 days' duration. Monthly mean temperature was $+1^{\circ}$ F. Precipitation frequent and averaged 0.2 in. above normal. Soil remained wet most of month. Downy mildew reported in Georgia on the 20th.

March temperatures about normal, precipitation 1.5 inches below normal, winds more or less prevalent. A warm period of 5 days occurred at Lumberton (17th-21st) when maximum temperature averaged 75° and minimum 54° . The disease appeared in this section on the 21st. Conditions favored crops and farm work. Tobacco beds generally in good condition at end of month with few, widely separated infected beds in southeastern part of State.

April temperatures, although averaging normal, were variable with prolonged cool rainy periods. Rains frequent and well-distributed. Average rainfall 0.5 inch above normal. Conditions favored good to excellent advance of early truck and fruits. Prolonged cool, rainy periods were conducive to much damage by downy mildew. Producers had greatly increased the area and numbers of beds; no spraying attempted; plants, in general,

not transplanted until signs of recovery were seen. These factors finally enabled the contemplated acreage to be transplanted. Considerable tobacco transplanted in the eastern counties during the month. Downy mildew appeared in the Piedmont section on the 7th.

May was the warmest experienced in the State in 37 years. Temperatures, above normal on 25 days, averaged $+3.9^{\circ}$ F. for the month. Rainfall above normal (0.4 inch), reasonably well-distributed throughout month. In some parts of the Piedmont, however, rain was needed at the close of the month. Progress of crops, fair to good. Sudden coming of warm weather checked downy mildew, especially in Piedmont section, where it generally appeared May 1. In many localities in this section the disease appeared in nearly all seed beds on virtually the same date. By the 22nd the disease was effectively checked in the more northerly sections, extending to Blackstone, Va.

As mentioned above, downy mildew caused considerable damage to tobacco seed beds during the very prolonged cool, cloudy, rainy periods of April. Producers had benefited by the 1932 experience. They had greatly increased seed bed areas, were beginning to locate the beds in better drained and more exposed sites, had refrained from spraying, and, in general, awaited signs of recovery of the seedlings before transplanting. These improved practices permitted the entire acreage to be planted, and one of the best tobacco crops of recent years was produced. There was no indication that downy mildew was present in the fields nor that the disease caused any bad effects on subsequent growth or quality of the crop.

In 1934. January quite mild until 28th, when most rigorous cold wave in 10 years occurred. Precipitation well-distributed throughout the State but was 1.5 inches below normal. Since the previous months were dry the stream flow was the lowest on record for January.

February was the coldest in the past 29 years, with a mean minimum temperature 6.2° F. below normal. Hard freezes on 8th, 10th, 18th, 27th, and 28th, with sleet and glaze on 25th and 26th, causing severe damage to forests and electric lines. Truck crops, small grain, and tobacco seedlings damaged. Many seed beds reseeded. Precipitation remained below normal; stream flow lowest on record for February.

Weather continued cold throughout March, temperatures averaging 2.0° F. below normal. Hard freeze on 11th and 12th. Precipitation 1.7 inches above normal; only 13 clear days during month. Little opportunity for preparation of soil for spring planting because of cold weather. Growth of tobacco seedlings retarded, by end of month they were 2 to 3 weeks behind normal condition. From March 3-8, at Lumberton, N. C., average maximum temperature 77° F. and the minimum 56° F. No primary infection at this time because previous cold weather had permitted little or no germination

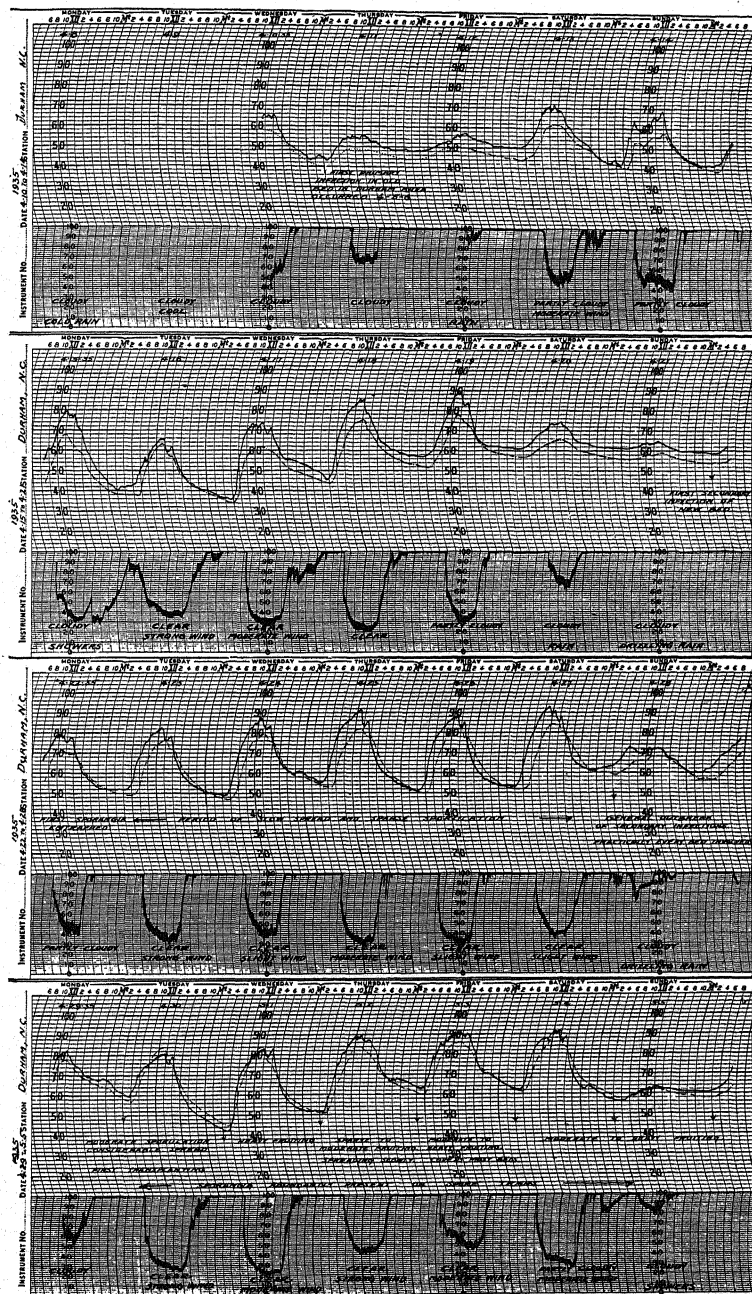


FIG. 4. Continuous record of air temperature, soil temperature, and air humidity within a seed bed at Durham, N. C. for the period Apr. 10-May 5, 1935.

and growth of tobacco seedlings. Minimum temperature for the remainder of the month, except on the 22nd, 27th, and 28th, ranged from 21° F. to 44° F.

April temperature very variable. Month began warm, and soil wet; month closed with cool weather and fields rather dry. Rainfall slightly above normal, poorly distributed. Two rainy periods, April 5-10 and 16-20. Growth of tobacco seedlings approximately 2 weeks behind the normal in all sections. From March 31 to April 8, average minimum temperature, 50° F. at Lumberton, N. C. At McDonald, N. C., self-recording instruments within seed bed showed average maximum of 85° F. and average minimum of 55° F. Primary outbreaks of downy mildew appeared in this area from April 4 to 9. The disease appeared in Wilson county on April 12, and in Durham, Vance, Guilford, Stokes, and Surry Counties on the 23d. Transplanting of tobacco in eastern portions of State about 20 days later than usual on account of downy mildew and unfavorable weather.

Average temperature and precipitation for May was normal except for marked warm, cool, dry, and wet periods. The warmest period came during first week of month and the coolest, during last week. Dry weather of late April extended into first half of May. It was necessary to water tobacco seed beds frequently during this period. Other crops suffered also from lack of water. General heavy rainfall occurred about May 15. Frequent rains and much cloudiness characterized period from 22nd to the end of month. Downy mildew generally prevalent over Piedmont section about May 4; peak of epiphytotic, about May 11. Disease spread slowly, because of dry weather; no serious damage occurred. Sporulation was renewed, however, during cloudy rainy days at end of month on seedlings remaining after transplanting. Less damage occurred in 1934 than in any other year in which the disease has been observed.

In 1935. January generally mild for first 22 days; then cool weather prevailed throughout the remainder of the month. Temperature 10° or more above normal during mild period and as much below normal during cold period. Precipitation averaged 0.5 inch above normal, and was well-distributed. Wet soil and low temperature during latter part of month prevented plowing.

Moderate temperatures during February excepting hard freeze on the last 2 days. Mean temperature was 0.7° F. above normal. Average precipitation 2.8 in. well distributed, was 1.3 in. below the average for a 48-year period. These conditions were very favorable for plowing and for germination of tobacco seed, especially in the southeastern portion of State. The late freeze reduced the stand of tobacco seedlings in a great number of seed beds in the Lumberton area.

March had no freezing temperatures throughout the flue-cured tobacco-

growing area after the first day. Monthly mean temperature 5.6° F. above normal. March 4-8, warm, maximum temperature averaged 74° F. and minimum temperature 50° F., at Lumberton. This condition is considered favorable for a primary outbreak of downy mildew, but no evidence of disease was found in this section until the 15th. This may be accounted for by the small size of tobacco seedlings at this time, and the thinning caused by the late February freeze. Seeds did not germinate until late February, and the first pair of true leaves was just unfolding. All four leaves were held well off the ground. Another warm period occurred in the Lumberton area March 14-22, when maximum temperature averaged 75° F. and minimum 52° F. Primary outbreaks occurred in this section from March 15 to 23, varying with localized conditions. Average precipitation 0.8 inch above normal. Weather favored rapid advance of vegetation, and season was at least 2 weeks earlier than in 1934.

April, rainy. Cold weather from 4th to 17th. Late April about normal. Average precipitation, 0.6 inch above normal. Low temperatures, frequent rains, and insufficient sunshine retarded growth and interfered with preparation of soil and planting. Some tobacco was transplanted in southeastern part of State before general outbreak on 11th. Some farmers had completed transplanting by 19th; in general, transplanting then only half done. Earliest infected beds then showed signs of recovery. Although nearly every bed in this section was infected, some quite badly, no reduction of acreage was incurred by reason of the disease. Primary outbreaks appeared in Piedmont section on 5th and 6th; general outbreak on 28th. Difficulty in obtaining plants experienced in some parts of the eastern portion of State because of repeated attacks by downy mildew fungus.

May temperatures averaged 1.3° F. below average for past 48 years. May 15th to 26th, cool, with insufficient sunshine for growing crops. Although the number of rainy days during May was above average, total rainfall was below normal in most tobacco-growing areas. Weather in general favorable for transplanting tobacco, and for continued sporulation by downy mildew fungus. Disease only moderately destructive this season in seed beds, but appeared, in several localities in the Carolinas, on large plants in the field.

CLIMATOLOGICAL CONDITIONS IN SEED BEDS AND THEIR INTERPRETATION

The continuous records made in 1934 include those of the air temperature and of the relative humidity inside the seed beds. Those made in 1935 include those of the air temperature outside of the seed bed and of the air temperature, relative humidity, and soil temperature within the seed bed. The air temperatures outside of the seed beds are not shown on the charts, but are essentially the same as those of local Weather Bureau Stations. The

air temperatures within the seed beds usually are slightly in excess of those outside the beds.

The graphs (Figs. 1-5) record the essential features of the course of downy-mildew disease of tobacco as related to climatological factors in 1934 and 1935. Similar records are not available for 1931 and 1932, but a record of a portion of the season of 1933 was obtained, and has been published (*loc. cit.*). Certain features of the disease in 1931, 1932, 1933, mentioned in preceding paragraphs, are known, and will be utilized in substantiation of the conclusions drawn from the data in figures 1-5. It is believed that the interpretation of these data will be simplified if attention be directed separately to such sequential events in the course of the disease and the development of the pathogen as (1) primary infection, (2) secondary infection, (3) sporulation, (4) dissemination of sporangia and the general outbreak of the disease, and (5) the recovery from it. Conditions correlated with each of these events, during each season, will, therefore, be compared and contrasted so far as the available data warrant.

Primary Infection. For the purpose of this report it is arbitrarily agreed that infections are first apparent when sporangia have been produced. Then too, infections are regarded as primary if (1) they occur in seed beds sown the following year on the sites of old diseased seed beds, if (2) sporulation occurs in such beds several (9-17) days prior to sporulation in any new (first-year) seed bed in that locality, if (3) when sporangia are produced, sporulation occurs on the surfaces of the lower leaves of a very few seedlings, and if (4) hibernating oospores constitute the inoculum for these infections. They are regarded as secondary if they occur 9-17 days after sporangia are known to have been present on primarily infected plants in the vicinity. Secondary infections may, therefore, occur (1) in old beds in which sporangia are first produced on primarily infected plants, (2) in new beds near primarily infected old ones, or (3) in old beds in which no primary infections occurred. Secondary infections in primarily infected old beds account for the increase in the number of affected seedlings in those beds.

The incidence of outbreaks, whether primary, secondary, or general, dates from the visible evidence of infection, *i.e.*, the production of sporangia.

The dates of occurrence of primary outbreaks, and also the first recurrence of the disease in 1931, are assembled for convenience in table 1.

The dates on which the disease first appears one year may be very different from those on which it first occurs another year. In explanation it seems reasonable to assume that (1) oospores causing primary infection must occur in the surface layer of soil, and that (2) the most important factor influencing the time of occurrence of primary infection is the maintenance of favorable temperature levels in this surface-soil layer for a sufficient length of time.

TABLE 1.—*Dates of the first occurrence of downy mildew of tobacco in North Carolina, and weather station records of temperatures for warm periods preceding primary outbreaks*

Year	Area	Date of earliest reported downy mildew occurrence	Time of occurrence of warm period	Duration of warm period (days)	Mean max. temp. ° F.	Range of max. temp. ° F.	Mean min. temp. ° F.	Range of min. temp. ° F.
1931	Lumberton	Apr. 27 (probably 19-25)	Apr. 14-22	9	78	70-84	50	43-57
1932	"	Feb. 23 (probably 15)	Feb. 10-14 Mar. 17-21 Mar. 31-	5 5	70 75	62-81 68-85	50 54	43-60 45-62
1933	"	Mar. 21	Apr. 8	9	82	65-89	50	42-58
1934	"	Apr. 4-9	Apr. 15-21 Mar. 14-22	7 9	69 75	56-78 64-79	51 52	43-59 47-58
1934	Durham	Apr. 23	Mar. 28-	7	72	64-84	48	43-52
1935	Lumberton	Mar. 20-23						
1935	Durham	Apr. 4-6						

In 1935, at McDonald, a record of soil temperature of the surface layer was obtained for a 22-day period prior to the primary outbreak in the seed bed containing the recording instruments. Examination of this record of soil temperatures at McDonald reveals the fact that a period of several days, in which the minimum soil temperatures were maintained at or above 50° F., preceded the primary outbreak. It must be borne in mind that this record shows the temperature conditions that obtained in the seed bed in which the instruments were kept. Temperature conditions in other seed beds in the same area most certainly differed, dependent upon such factors as exposure, drainage conditions, type of soil, thickness of seed-bed covers, and protection afforded the beds by surrounding forests. In consequence, not all primary outbreaks may necessarily occur in a given locality on one and the same day.

Unfortunately, primary infection occurred, in 1935, in the selected bed near Durham, prior to the installation of the instruments. The week preceding the outbreak was, however, characteristically mild.

Soil temperatures are not known for 1931-1934. A comparison of the temperatures of the air within the seed beds for March, April, and May, 1935, with the soil temperatures for the corresponding period shows that they rather closely approximate each other. The maximum seed-bed air temperatures average 5° F. higher than the maximum soil temperatures, and the minimum air temperatures average 3° F. higher than the minimum soil temperatures. In 1934, as shown in figures 1 and 2, several days of warm weather preceded primary outbreaks both in the area near McDonald and in that near Durham. The average minimum seed-bed air temperature at McDonald during this critical period was 55° F., and that at Durham was 53° F. The minimum soil temperatures during this period in 1934, as indicated by comparison with temperature conditions within seed beds in 1935, probably were about 50° F.

The records obtained during 1933 do not cover the period preceding the occurrence of primary infection. The temperatures recorded by Weather Bureau Stations for the warm period preceding primary outbreaks in 1933, and 1932, and for the period in which the first outbreak occurred in 1931, show striking similarities. The temperature conditions recorded by the Weather Bureau for these mild periods are compared in table 1, with similar data for 1934 and 1935.

The outstanding phenomenon indicated by these data is that primary outbreaks occur during or following a warm period in which minimum temperatures of approximately 50° F. are maintained for several days. These periods may be characterized as "warm spells." The weather during such spells may be either clear and dry, or cloudy and rainy, without apparent effect on the occurrence of the disease. The primary outbreaks occurring earliest in the season, in the southeastern portions of the State, have taken place during or shortly after clear, bright, warm weather.

Primary infection may not occur during the first warm spell in winter or very early spring. Although seedlings of all ages are known to be subject to attack under controlled environmental conditions, those in seed beds do not become infected until the leaves extend horizontally and the lower ones come in contact with the soil. This may explain why warm periods occurring shortly after the germination of the tobacco seed fail to induce primary infection.

(2) *Secondary Infections.* Examination of figures 1-5 shows that secondary infections were first evident in seed beds near primarily infected ones within approximately two weeks after the first sporulation in primarily infected beds. Our observations show that repeated crops of sporangia are being produced during this period in primarily infected beds, and that a large proportion of the seedlings have become involved.

In 1934, near McDonald, the interval between the occurrence of primary outbreaks and the earliest occurrence of secondary outbreaks in near-by beds was 12 days. The weather for this period was predominantly clear, with moderate to strong winds. The maximum temperature ranged from 63° F. to 94° F. and the minimum from 36° F. to 60° F. The corresponding interval near Durham was 11 days, with the same general type of weather. Maximum day temperature was higher, however, ranging from 75° F. to 100° F. with minimum night temperature from 40° F. to 57° F. The soil generally was rather dry.

In 1935, near McDonald, the interval between the occurrence of primary outbreak and the earliest outbreak in near-by beds was 13 days. The weather was predominantly partly cloudy with some rain, broken by clear days accompanied by mild to strong winds. Maximum temperature ranged from 65° F.-95° F. and minimum from 46° F.-66° F. The corresponding interval, near Durham was 17 days. Weather was extremely variable during this period. Cool rainy periods, cloudy and partly cloudy days, clear days with moderate to strong winds, and periods of drizzling rain were experienced. Maximum temperature ranged from 53° F.-89° F., and minimum temperature from 36° F.-61° F.

The mean maximum temperature for these intervals during the 2 seasons is 78° F., with a range of 53° F.-100° F. The mean minimum temperature is 51° F., and the range 36° F.-66° F. During these periods, the air within the seed beds was saturated for an average of 12 hours per day, and dew remained on the foliage several hours after sunrise nearly every day. Since it is known that infection occurs when viable sporangia are maintained in films of water for several hours, and that saturation conditions prevailed for an average of 12 hours each day, it is concluded that secondary infection may take place during any general type of weather prevalent at this period of year.

TABLE 2.—*Weather conditions during production of sporangia following the general outbreak of the disease*

Year	Period of sporulation following general outbreak (days)	Mean maximum temperature ° F.	Range maximum temperature ° F.	Mean minimum temperature ° F.	Range minimum temperature ° F.	Mean minimum rel. humidity (%)	Range minimum rel. humidity (%)	Av. duration of saturation periods (hrs.)	Range of saturation (hrs.)	General weather conditions
1933	10	81	62-90	61	52-68	77	54-86	23	12-64	Cloudy, Rainy, Humid and Wet
1934	16	93	67-106	57	47-67	43	28-78	12	6-19	Clear, intense Sunshine, Dry
1935	15	83	64-92	58	42-64	46	22-80	12	3-17	Consecutive days of clear, windy and cloudy rainy weather

(3) *Sporulation*. In a previous account² it was pointed out that the production of sporangia begins in early morning and is usually completed by sunrise, and that it is most profuse during periods of cloudy, rainy weather, when the sky is overcast for long periods. It also is true that sporulation can occur but may be less abundant during other kinds of weather. The period during which sporangia may be produced in any locality may vary in length from year to year, as shown by our later observations. The factors that govern this phenomenon are summarized in table 2.

In explanation of these data it may be recounted that, in 1933, near Oxford, the duration of the period from the general outbreak of downy mildew in new beds until sporangia were no longer produced was 10 days, 7 of which were cloudy and rainy. The average maximum temperature during this period was 81° F., and the minimum ranged between 52° and 68° F. Sporulation was abruptly terminated after a day when the maximum was 90° F. and the minimum was 68° F. In the same year, near Blackstone, Va., almost no sporulation occurred on the morning following a day when the maximum temperature was 97° F. and the minimum night temperature was 66° F. No sporangia were formed subsequently in this area.

In 1934, near Durham, the duration of the corresponding period was 16 days, on 4 of which there was precipitation, and 10 days were clear. Sporulation was sparse on the first 6 days of the period, days that were unusually hot, with maximum temperature of 95° F. or above. This hot spell was followed by 3 partly cloudy days, with maximum temperature near 90° F. and minima of 63°, 53° and 52° F., respectively. Abundant sporulation occurred during these three days. Minimum temperatures ranged from 47° to 67° F. during the remainder of the period and sporulation was sparse.

In 1935, near Durham, the duration of this period of sporulation was 15 days, 7 of which were clear, and on 6 of which it rained. The variability in weather this season was associated with variableness in amount of sporulation. Brief periods of abundant, moderate, and sparse sporulation were intermingled. The maximum temperatures varied from 64° to 92° F. and the minimum from 42° to 64° F.

When consideration is given to temperature conditions prevalent from the advent of sporangia on primarily infected plants to cessation of their development, which includes the sporulation period whose conditions are given in table 2, it is concluded that moderate to abundant sporulation occurs at minimum temperatures ranging from 42° F.—63° F. and sporangia are produced most abundantly at approximately 56° F. If the minimum temperatures are outside this range, either less than 42° F. or greater than 63° F., sporulation is sparse. Little if any production of sporangia occurs above 68° F. or below 36° F.

² *Loc. cit.*

As regards the influence of humidity factors on sporulation, attention may again be directed to the data in table 2. During the 10-day interval in 1933, the air within the seed bed remained saturated for periods ranging from 12 to 64 hours in length, or an average of 23 hours. Cloudy, rainy weather prevailed, with a minimum relative humidity of 80 per cent or more for the entire 10 days except for approximately 3 hours. The production of sporangia did not cease at sunrise, but evidently continued throughout certain of these days. As a consequence, the progress of the disease was more rapid than in either of the two following years.

During the 16-day period, in 1934, the periods of saturation of the air within seed beds averaged 12 hours and ranged from 6 to 19 hours. The weather was predominantly clear, with intense sunshine. Minimum relative humidity averaged 43 per cent for the 16 days and varied from 28 to 78 per cent, except for approximately an hour, when it was 80 per cent. Sporangia were sparsely produced, except during 3 partly cloudy days that followed a drizzling rain. The progress of the disease was slower and less damage resulted than in 1933 or in 1935.

In 1935, during the 15-day period, the air within seed beds remained saturated for average periods of 12 hours, the length ranging from 3 to 17 hours. Consecutive days of clear, windy weather and of cloudy, rainy weather occurred. Minimum relative humidity averaged 46 per cent and varied from 22 to 80 per cent. It was 54 per cent or less on 12 of the 15 days. The progress and destructiveness of downy mildew was intermediate between that in 1933 and that in 1934.

From comparison of the data (Table 2) on humidity factors in 1934 and 1935, one might anticipate that the amount of sporulation and the destructiveness of the disease should be quite alike. As a matter of fact, sporulation was more abundant and downy mildew more destructive in 1935 than in 1934. These differences are attributable in part to the large proportion of days with high temperature and intense sunshine in 1934. It may be recalled that a temperature of approximately 85° F. or above, is lethal to sporangia, and that exposure of sporangia to direct sunlight for short periods also is lethal (*loc. cit.*). If comparison be made of humidity factors in 1933 with those in 1934 and 1935, it will be apparent that long periods of saturation and overcast skies favor sporulation.

(4) *Dissemination of Sporangia and the General Outbreak of the Disease.* Air currents are known to be the most important agent for dissemination of sporangia.

The dissemination periods may, for convenience, be divided into two, primary and secondary. The primary period is that interval between the earliest occurrence of sporangia in primarily infected beds and the earliest sporulation in new beds in the same locality. The secondary period extends

TABLE 3.—Sequence of outbreaks of downy mildew and intervening intervals

Year	Locality	Dates of primary outbreaks	Elapsed time (primary dissemination period) (days)	Dates of secondary outbreaks	Elapsed time (secondary dissemination period) (days)	Dates of general outbreak	Total elapsed time from first fruiting in primarily infected bed to fruiting in all beds (days)	Date when sporangia first trapped
1933	Oxford	Apr. 7				Apr. 30– May 2	25	Apr. 25
1934	McDonald Durham	Apr. 4–5	12	Apr. 16	12	Apr. 28	24	Apr. 9
		Apr. 23	11	May 4	10	May 14	21	May 3
1935	McDonald Durham	Mar. 20–23	13	Apr. 2	9	Apr. 11	22	Mar. 31
		Apr. 5–6	17	Apr. 22	6	Apr. 28	23	Apr. 22

from the date of the secondary outbreaks to the date of the general outbreak. The inoculum disseminated during this secondary period originates both in primarily and secondarily infected beds, and is, therefore, greater in amount than occurs during the primary period.

These periods of dissemination and other pertinent facts for 1933, 1934 and 1935 are shown in table 3.

In 1935, near McDonald, the primary dissemination period lasted 13 days. Sporangia were first entrapped on March 31. Infections probably arose from inoculum disseminated on March 25-27, when windy weather prevailed. A few seed beds at widely separated points throughout the area evidenced infection on April 2. The secondary period of dissemination lasted 9 days and terminated on April 11. The weather was cool and rainy, interspersed with dry, windy days.

The primary dissemination period near Durham lasted 17 days. A few plants in a small number of beds evidenced infection at the end of this period. The fact that no sporangia were found on the spore traps during this period might be interpreted to indicate that conditions were not favorable for dissemination. There were, in fact, few sporangia to be disseminated because low night temperatures interfered with their production. On April 20, 21, and 22 abundant sporulation took place and the 5 clear, windy days that occurred in succession thereafter were ideal for dissemination. On April 28, the day following these 5 days, the general outbreak of the disease occurred. Several small groups of plants in every bed evidenced infection on this date.

As shown by tests with spore traps, sporangia were being disseminated in abundance near Durham, in 1935, from April 30 to May 6 and May 8 to 9. The weather was clear with gentle winds, and sporangia were being produced in abundance during these two intervals.

In 1934, near McDonald, the primary period of dissemination terminated on April 16, 12 days after the primary outbreak. Sporangia were first entrapped on April 9. During this period (April 4-16), temperature and humidity conditions were conducive to moderate production of sporangia, and protracted winds, varying in intensity from a mild breeze to a gale, facilitated transport of sporangia. Consequently, a large number of beds were infected at the end of the primary period. The weather of secondary period, April 17 to 28, favored dissemination but was unfavorable for sporulation.

In 1934 the primary dissemination period near Durham terminated May 4, and included an interval of 11 days. Sporangia were first entrapped 10 days after the primary outbreak. The maximum temperature of 100° F. on April 27, probably was lethal to sporangia that may have formed early that morning. The 6 days that followed, with an average maximum temperature

of 82° F. and an average minimum of 51° F. favored sporulation. The then prevalent winds favored dissemination. As a result, groups of diseased plants were found present in about 80 per cent of the beds examined on May 4. The area occupied by diseased plants in these beds increased slowly during the next 10 days, and the infections had meanwhile become evident in the remaining seed beds near Durham.

In 1933, near Oxford, the first dissemination period lasted from April 7 to approximately April 30. Sporangia were first entrapped on April 25. The continuously cloudy, rainy weather of the week from April 15 to 21 was excellent for abundant production of sporangia but inhibited their transport. The following week favored both production and dissemination. In consequence, downy mildew appeared in many seed beds on April 30, and the general outbreak occurred two days later. The primary and secondary periods of dissemination were, therefore, fused because of the lack of disseminating conditions during the normal primary dissemination period.

It is, therefore, apparent that in each of these seasons an interval of about 3 weeks elapsed between the occurrence of sporangia on primarily infected plants and the general outbreak of the disease. This apparently is not fortuitous but is presumably related to the fact that a period of about 7 days is required for the completion of the cycle of the sporangial stage. On this basis sporulation on new beds might be expected to occur one week after that on primarily infected beds. It appears, however, that infection from this inoculum is confined to seedlings near the primarily infected seedlings and in the same bed. The inoculum produced on these additional plants is sufficient to initiate the secondary outbreak at the end of another week. This accounts for the occurrence of secondary outbreaks about 2 weeks after primary outbreaks. The general outbreak occurs about a week later, due to the greatly increased quantity of inoculum disseminated from widely distributed sporulating beds. Weather conditions may so affect sporulation and dissemination as to prevent secondary outbreaks from occurring about 2 weeks after primary outbreaks, as was the case in 1933; or, on the other hand, they may be such as to cause the disease to appear in a large number of beds at this time, as was the case in 1934.

It is concluded from these data that sporangia are disseminated during windy weather, which may be either clear or cloudy. Continued rainy weather, combined with long periods of saturation, although conducive to abundant sporulation, inhibits dissemination. The greatest quantity of inoculum is disseminated when abundant sporulation occurs during windy weather, or when windy weather follows immediately after heavy sporulation periods. If prolonged periods of high temperatures, intense sunshine, and drying winds obtain, few sporangia are disseminated, because such weather results in a dearth of inoculum. Only a few sporangia transported under such conditions are capable of producing infections.

(5) *Recovery from Downy Mildew.* The time required for downy mildew of tobacco to run its course varies from year to year, a fact no doubt related to the meteorological factors that modify sporulation. When the weather is unfavorable for production of sporangia, the lesions may appear as yellow flecks. When sporulation is sparse, there may be successive scant crops of sporangia on new tissue or at the margins of old lesions on several successive mornings. When sporulation is abundant, one or two crops of sporangia are produced, after which the invaded tissues collapse rather quickly. Our observations show that plants begin to recover when sporulation ceases. A summary of weather conditions that prevailed during periods in which sporangia were not produced in 1933, 1934, and 1935 is shown in table 4.

It may be noticed that in the areas considered in table 4 the period extending from the general outbreak to the beginning of recovery lasted 10 days in 1933, 16 days in 1934, and 15 days in 1935. In 1933 the average minimum night temperature during the period of recovery was unfavorable for sporulation. In 1934, both the night temperature and relative humidity conditions were favorable during this period. In 1935 a period of sparse sporulation of 5 days duration interrupted the 2 periods in which sporangia were not produced. During both periods the average maximum temperature approximated 80° F., the average minimum temperature approximated 57° F., and the average duration of periods of saturation was 18 hours. On the basis of the conclusions previously drawn regarding the influence of climatic factors on sporulation, one would have expected the weather conditions, in both periods, to favor abundant production of sporangia.

It seems reasonable to conclude from the data shown in table 4 that the initiation of recovery from downy mildew is not primarily conditioned by weather. Further evidence in support of this conclusion is afforded by the fact that seedlings in one end of a bed may be recovering while the disease is not visibly present on those in the other end, and while those intermediately located are suffering intermediate stages of the disease. This condition is most noticeable in primarily infected seed beds. No satisfactory explanation for this behavior can be given at this time. The factors that initiate recovery, however, seem not to be external but internal as regards the seedlings. If immunity is conferred to tobacco seedlings as the result of downy mildew infection, it must be regarded only as temporary. In 1935, for example, sporulation occurred from May 19 to 23, on plants on which the production of sporangia had ceased for a period of a week prior. The reinfection of seedlings after they are well on the way to complete recovery has been noted by various investigators. The fact is equally well-known that plants are subject to attack after having been transplanted sufficiently long to have become well-established in the field.

Although weather conditions are not of primary importance in initiating recovery, they can modify the rate of recovery. Warm, clear days, followed

TABLE 4.—*Weather conditions during recovery from downy mildew*

Location and year	Interval from gen- eral out- break to first cessa- tion of sporulation (days)	Periods of no sporu- lation	Mean max. temp. (°F)	Range max. temp. (°F)	Mean min. temp. (°F)	Range min. temp. (°F)	Mean min. humidity (%)	Range humidity (%)	Average duration of satu- ration (hrs.)	Range saturation (hrs.)
Oxford, 1933	10	May 12-16	91	87-94	68	67-69	87	80-92	21	18-44
Durham, 1934 ..	16	May 20-27	88	74-98	60	51-66	57	45-70	13	10-16
Durham, 1935 ..	15	May 12-19 May 23-30	79 81	61-90 60-89	58 56	50-66 51-64	52 45	35-90 38-52	18 18	13-37 13-38

by warm nights inhibit sporulation, are lethal to sporangia, and promote the growth of the tobacco seedlings. There is thus a basis for the impression commonly held that warm sunny weather causes infected seedlings to recover.

GENERAL SUMMARY

This report deals with climatic conditions that occurred in North Carolina in 1931-1935 as related to the course of the downy-mildew disease of tobacco.

The weather for each year is presented by months, as a background for an understanding of differences in time of occurrence and course of the disease.

Primary outbreaks do not occur on corresponding dates in successive years. They occur during or immediately after periods of warm weather, (1) when the minimum temperature of the surface layer of soil has been maintained at or above 50° F. for several days, and (2) when the seedlings are of sufficient size for the lower leaves to come in contact with the soil. They occur irrespective of the character of the sky or of rainfall.

Secondary infection may occur during any general type of weather prevalent at this season of the year, because dew accumulates on the leaves of the seedlings each night. Secondary outbreaks occur approximately 2 weeks after primary outbreaks, and are conditioned to some extent by the weather as it affects sporulation and dissemination.

Sporulation is favored by long periods of saturation at times when the sky is overcast. Abundant sporulation occurs within a range of temperature from 42° F. to 63° F. and is most abundant at 56° F. If the temperature is outside this range, either less than 42° F. or greater than 63° F., sporulation is sparse. Few if any sporangia are formed above 68° F. or below 36° F. Maximum temperature above 90° F., accompanied by intense sunshine, inhibits abundant sporulation even when night conditions are favorable.

Windy weather, either clear or cloudy, is favorable for the dissemination of sporangia. The greatest amount of inoculum is disseminated when windy weather occurs during, or immediately after, periods of abundant sporulation. The general outbreak of downy mildew in any locality occurs about 3 weeks after the primary outbreak. The primary period of dissemination is approximately 2 weeks long and the secondary period approximately 1 week.

Weather conditions are not of primary importance in initiating recovery. Recovery appears to be initiated by factors within the plant. Tobacco seedlings recover most rapidly, however, during warm clear days, and warm nights.

DUKE UNIVERSITY,
DURHAM, N. C.

FURTHER STUDIES ON DOWNY MILDEW OF TOBACCO

FREDERICK A. WOLF, RUTH A. MCLEAN, AND LAWRENCE F. DIXON¹

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INTRODUCTION

Downy mildew is a disease of tobacco seedlings of major importance to all growers of flue-cured tobacco within the United States and to others in portions of the areas devoted to the culture of Burley tobacco. The results of our investigations dealing with certain features of this disease and with its causal agency have been stated and interpreted in previous reports (16, 6, 18, 19). Additional experimental data, secured during the past year, and observations made in the field during the past 4 years, are here assembled to supplement the present knowledge of this disease. These data and observations partly confirm our previous findings and are corroborative of conclusions previously drawn.

Evidence in an earlier report (6) led to the conclusion (1) that oospores serve as inoculum for primary infections and (2) that seed beds sown on the sites of beds devoted to tobacco seedlings the preceding year constitute the loci of primary infection for downy mildew. The probable correctness of these conclusions is supported by the fact that, in 1934, the disease was found to develop earlier in seed beds located on sites occupied by beds during the previous year than in those sown on new sites. The earliest development of the disease in every locality occurred, in every instance, in beds sown on the sites of old beds. Furthermore, sporulation in primarily infected beds occurred prior to the time that sporangia could be entrapped from the air in the same locality. Since the probability remained, however, that the findings relating to the initiation of downy mildew in 1934 might not be duplicated in another season, or in other areas, the investigations were continued throughout the season of 1935. The same methods of investigation already described (6) were followed in this portion of the work.

INITIATION OF PRIMARY INFECTIONS

Two field stations were established this season, where daily observations on the initiation of downy mildew and on the progress of the disease were made. One was located in Robeson County, near McDonald, North Carolina, and the other in Durham County, near Durham. These observations,

¹ The writers gratefully acknowledge the sustained interest of Dr. P. M. Gross in the investigations of downy mildew of tobacco, and are appreciative of his criticisms during the preparation of this report.

begun March 1 and continued till May 31, were supplemented, as much as time permitted, by surveys involving areas in Horry County, South Carolina, and in Wake, Granville, Orange, and Vance Counties, North Carolina. When downy mildew became generally prevalent in the environs of McDonald, observations in that locality were discontinued. Consideration was given not only to the dates of appearance of the disease in beds on old sites in comparison with those on new sites, but also to the prevalence of air-borne sporangia as indicated by spore traps (Fig. 1, A), and by the time of occurrence of secondary infections in the vicinity of primarily infected beds (Table 1).

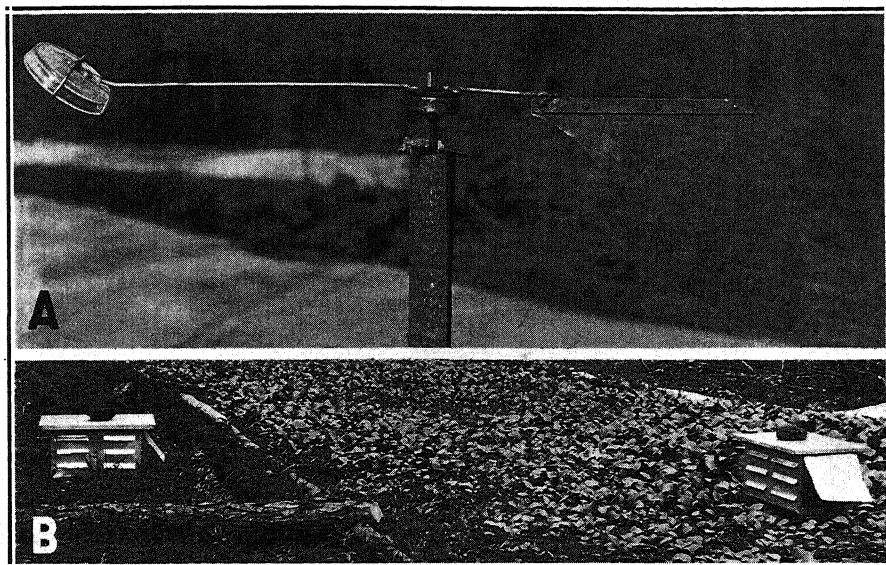


FIG. 1. A. Spore-trap mechanism, consisting of a roller bearing and a shaft at one end of which the dishes were attached and at the other a vane. By this means the adhesive inner surface of the dish was continuously directed toward the wind. B. Ventilated shelters for the protection of the Friez instruments used in making continuous records of soil temperature, air temperature, and air humidity. The shelters were insulated with Cello-tex pads.

Attention first should be directed to the fact that, without exception, the earliest infections again occurred in beds located on sites occupied by diseased beds during the preceding year. Among the 114 beds on old sites, under observation in 1935, primary infection occurred in 31. In 1934, primary infection occurred in 37 of the 106 old beds. In explanation of the proportional differences in number of primary infections in these two seasons it may be pointed out that in the Durham area a special effort was made in 1935 to thoroughly sterilize, by burning, all old bed sites that

TABLE 1.—Summary of data on the effect of site of seed beds upon the incidence of downy mildew, and on the occurrence of air-borne sporangia

Observations	Areas			
	Robeson Co., N. C.	Horry Co., S. C.	Durham Co., N. C.	Total
Beds on old sites, number examined	61	30	23	114
Beds on new sites, number examined	59	16	49	124
Beds on old sites, area (sq. yds.)	6,480	2,040	4,210	12,730
Beds on new sites, area (sq. yds.)	7,420	4,160	8,130	19,710
Beds on old sites, number of primary infections	14	12	5	31
Beds on new sites, number of primary infections	0	0	0	0
Beds on old sites, dates of primary infection	Mar. 15–23	Mar. 15–20	Apr. 4–6	
Beds on new sites, date of earliest occurrence of downy mildew	Apr. 2	Mar. 28	Apr. 22	
Date when downy mildew was generally prevalent	Apr. 11	Apr. 11	Apr. 28	
Number of spore-traps exposed	288		364	652
Area of surface of spore-traps, sq. cm.	16,416		20,748	37,164
	38		61	91
Number of days on which spore traps were exposed	(Mar. 1–Apr. 8)		(Apr. 1–May 31)	
Number of days on which sporangia were entrapped	4		40	44
Date on which sporangia were first entrapped		Mar. 31	Apr. 22	

were employed. The percentage of the beds on old sites that evidence primary infection in spring is, however, of less significance than the fact that primary infection occurs in such beds, and that infections in beds on new sites did not occur prior to the outbreak of the disease in old beds in that locality.

The meteorological conditions of 1935 were more favorable than those of 1934, for the earlier development of tobacco seedlings and of other vegetation. Primary infections of downy mildew were evident approximately 2 weeks earlier than in 1934, and could be found in any locality when the flowering dogwood, *Cornus florida*, came into blossom. The dates of primary outbreaks in Robeson County and the contiguous area in Horry County extended over the period March 15–23; and in Durham County, April 4–6; the corresponding dates, in 1934, for these areas, were April 4–9, and April 23–24, respectively. Our observations show that primary infections were first evident, on March 29, in Greene County and in southern Wake County, areas in which the advance of the season is intermediate between that in the vicinity of McDonald and of Durham. This indicates that, concomitant with the advance of the season, there was

again, as in 1934, a northward progression in the occurrence of primary outbreaks.

Downy mildew first occurred, this year, in the Georgia area, on March 7. Since its earliest occurrence in South Carolina and North Carolina was only a week later, there would appear to be little likelihood that inoculum from Georgia initiated the outbreaks in these States.

Emphasis also should be placed upon the highly localized character of primary infections, previously pointed out (6), in differentiating between the appearance of a primary and a secondary infection. In primary infections a crop of sporangia may be found upon the lower surface of a single lower leaf of a lone seedling or upon the lower leaves of a group of 3 or 4 plants. The discovery of primary infection in a seed bed of several hundred square yards can reasonably be said, therefore, to be difficult. When, however, sporangia on primarily infected plants have given rise to secondary infection, and seedlings in an area a foot or more in diameter have become affected, the locus of infection is readily apparent.

In a given seed bed there were usually very few (1 to 5) loci of primary infection. It is not known why primary outbreaks are so highly localized rather than widespread, in view of the probability that myriads of oospores must be formed in every seed bed, and, on decay of the leaves, must be liberated in the soil of that bed. Many of them may be destroyed by biotic agents. Evidence will be presented subsequently to show that only a small proportion of the oospores retain their ability to germinate. It is probably for these reasons that a few, infected seedlings, or but one, may be present in a bed containing thousands of seedlings.

Another important feature of the observations summarized in table 1 is the intervals that elapsed between the time that primary infections are apparent and the earliest outbreak of downy mildew in near-by seed beds. This interval was about 10 to 14 days. It may be pointed out also that an interval of approximately 3 weeks elapsed in both 1934 and 1935 between the date when the first crop of sporangia was produced in primarily infected beds and the date when the disease was beginning to be generally prevalent in that locality. Both in the earliest occurrence of infection in secondarily infected beds and in the general outbreak of downy mildew, the fairly well defined periods found are undoubtedly due to the fact that the development of the sporangial stage (including germination, penetration, incubation, infection, and sporulation) usually requires approximately a week. The number of sporangia borne on primarily infected plants is small. As a result of secondary infection, however, in these primarily infected beds, the inoculum increases so that, within 10 to 14 days, an occasional seed bed near at hand is infected. The amount of

inoculum originating in these scattered beds is sufficient to cause a generalized outbreak after an additional week, and the disease will, soon afterwards, have attained epiphytotic proportions.

The spore traps consisted of shallow dishes (Fig. 1, A) whose inner surfaces were coated with glycerine. Sporangia were entrapped on 44 days of the 91 on which spore traps were exposed. The series of dishes exposed from April 22–May 31 showed that dissemination was occurring throughout the entire period. At 2 periods, namely, April 30–May 5 and May 8–9, sporangia were abundantly present in the air. The production of abundant crops of sporangia on the seedlings occurred during these intervals. Sporangia of other species of downy mildew were present on the plates throughout most of April and May. This was expected to occur, since the following species of downy mildew were collected on weeds growing within the beds or in the area closely surrounding them: *Peronospora alsinearum* Casp. on *Cerastium viscosum* L., *P. halstedii* Farl. on *Ambrosia artemisiifolia* L., *P. geranii* Pk. on *Geranium carolinianum* L., *P. parasitica* (Pers.) Fr. on *Cardamine pennsylvanica* Muhl., *Basidiosporea entospora* Roze et Cornu on *Erigeron canadensis* L., and *Bremia lactucae* Regel on *Lactuca canadensis* L. Cabbage seedlings commonly are grown along the border of tobacco seed beds in North Carolina and generally are parasitized by *Peronospora parasitica*. The sporangia of all of these, except *P. alsinearum*, can fairly certainly be differentiated from those of *P. tabacina* on the basis of size and shape. The significant result of the experiments with spore traps is that sporangia were entrapped, regardless of the particular species; that those of *P. tabacina* were most abundant on the dishes at times when sporangial production was most abundant on the tobacco seedlings, and that sporangia were not present in the air until a considerable period after primary outbreaks of downy mildew had taken place.

GERMINATION OF OOSPORES

Observations on germination of the oospores of the Peronosporaceae are limited. They appear to be confined, in the case of the genus *Peronospora*, to those of McKay (15) in his studies of the onion mildew, *P. schleideni* Ung. He placed affected leaves containing large numbers of oospores in a box that remained exposed in a garden. This material was tested from time to time by placing a portion of it in water, with the result that after 4 years an occasional oospore germinated. Five months later about 1 per cent of a lot of oospores that had been kept in water for 11 days were noted to have formed a stout tube that penetrated the enveloping folds of the persistent oogonial wall.

In testing the germination of oospores of *Peronospora tabacina*, decaying diseased leaves, collected in May, 1933, were placed intermittently in cold storage and in the laboratory. In the following December an occasional oospore produced a germ tube when bits of this material were macerated and kept in drops of water on microscopic slides. Other oospore-bearing material was collected in 1934, a portion was air-dried and kept in the laboratory; another portion was mixed with sand in a porous earthen vessel and then buried in the soil of an old bed. During the winter and spring of 1935, samples from each portion were taken periodically and maintained in drops of water to permit of germination at temperatures ranging from 36° F. to 75° F. Since less than a dozen oospores were observed to germinate as a result of these tests in which approximately several thousand oospores were employed, nothing definite is known about the environmental factors governing germination.

The first evidence of germination is the uniformly granular condition of the entire content of the oospore. The germ tube emerges through a cleft in the brown inner membrane and the clear outer oogonial envelope, or else it perforates this envelope (Fig. 2). The content of the germ tube is at first brown and granular, and of the same appearance as that of the oospore. As the tube continues to elongate, the contents become less

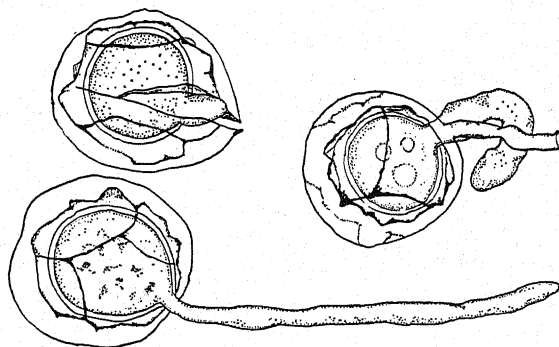


FIG. 2. Stages in germination of the oospores of *Peronospora tabacina*.

densely colored. The maximum length of this tube is about 4 times that of the diameter of the oospore.

The logical assumption, previously made (6), and supported by the current season's experiments, is that the oospores of *Peronospora tabacina* hibernate in the soil and constitute the inocula for the primary infections of tobacco seedlings. This assumption becomes the more cogent in view of the fact that the oospores have been observed to germinate. It is entirely in accord, moreover, with the opinion of mycologists generally as to the rôle of oospores in the life cycle of downy mildews. This is

borne out by the observations on downy mildew of grapes, *Plasmopara viticola* (B. et C.) Berl. et de Toni, (8), the downy mildew of cucurbits, *Peronoplasmopara cubensis* (B. et C.) Clinton (11, 14), the downy mildew of grasses, *Sclerospora graminicola* (Sacc.) Schroet. (9, 10), the downy mildew of onions, *Peronospora schleideni* Ung. (13, 15) and that of spinach, *P. effusa* (Grev.) Ces. (12). Unfortunately, penetration of tobacco seedlings by germ tubes from oospores of *P. tabacina* and the subsequent stages of infection have not been observed. It is, of course, highly desirable that these phenomena be observed and described, but the chances of their accomplishment appear remote when it is remembered that primary infection involves a few seedlings among a population of thousands, and apparently arises from only one or a few germinating oospores in seed beds in whose soil multiplied thousands of oospores are probably present.

SIZE OF THE REPRODUCTIVE STRUCTURES OF PERONOSPORA TABACINA

The measurements of the reproductive structures of *Peronospora tabacina*, as recorded by various investigators, do not agree. In no case have these records been based upon a sufficiently large number of measurements. For this reason a relatively large number of measurements of sporangia, oogonia, and oospores have been made, and the results have been assembled graphically in figures 3 and 4. Each graph shows the range in size and percentage of each size. Using freshly-formed sporangia, approximately 1,100 measurements of each dimension were made. More than 600

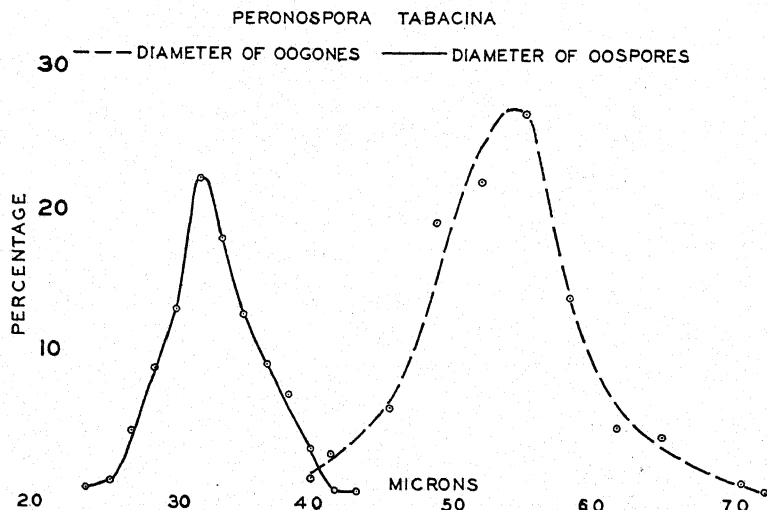


FIG. 3. Distribution of the diameters of approximately 600 oogonia and oospores of *Peronospora tabacina*.

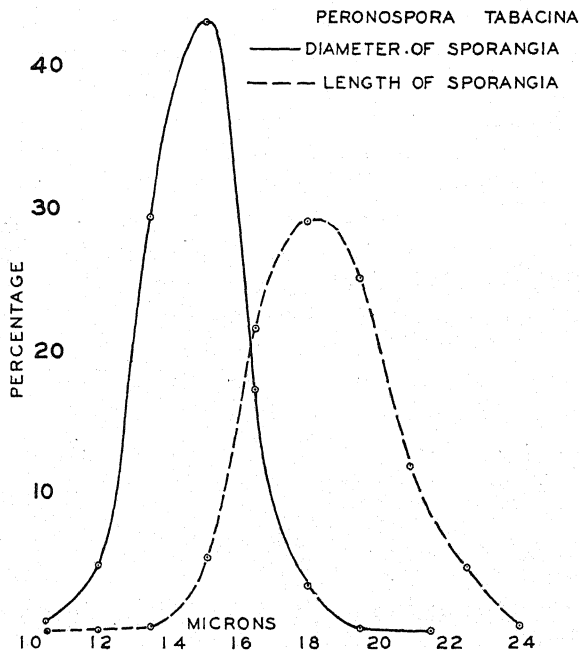


FIG. 4. Distribution of the lengths and widths of 1100 sporangia of *Peronospora tabacina*.

measurements of each structure were made in the case of oogonia and oospores. This material, from collections made in 1934, was mounted and cleared in dilute lactic acid.

From consultation of the results in figure 4, it may be seen that the range in length of sporangia varies from 10.5 to 24 μ , approximately 80 per cent being from 16 to 24 μ , with a mean length of 18.4 μ . The width of sporangia varies from 10.5 to 22 μ , approximately 85 per cent being 13 to 17 μ wide, with a mean width of 15 μ .

From figure 3 it may be observed that the oogonia range in diameter from 40 to 74 μ , approximately 75 per cent being from 47 to 57 μ , with 53 μ as a mean; and the oospores, from 24 to 43 μ , 65 per cent being 30 to 35 μ , with a mean diameter of 32 μ .

IDENTITY AND MORPHOLOGY OF PERONOSPORA SPP.² ON TOBACCO

Mycologists of the Old World and of North America have for years regarded the downy mildew fungus of tobacco as *Peronospora hyoscyami*

² The writers have examined specimens of *P. hyoscyami* de By., collected by Bakhtin, and of *P. nicotianae* Speg., both of which were loaned by S. F. Ashby, Kew Herbarium. *P. tabacina* from Australia was supplied by L. F. Mandelson, Brisbane, Australia. We acknowledge our indebtedness to both of these investigators.

de By., and that of South America as *P. nicotianae* Speg. (17). Although the sporangial stage of the former species was described in 1863 (4), its oogonial stage, collected in central Russia (Samara), was first described by Bakhtin (3) in 1926. The oogonia are 45 to 60 μ in diameter and are of the type that belong to the section Leiothecae. The oospores are yellowish brown, 30 to 44 μ in diameter, possess an irregularly folded perineum, 5 to 7 μ thick, and resemble very closely those of *P. tabacina*. The variations in measurements of sporangia are shown by the fact that those recorded by de Bary (4) are 13 to 24 \times 13 to 18 μ , by Bakhtin, (4) 20 to 27 \times 14 to 20 μ , and those communicated by Ashby³ are 21 to 28 \times 17 to 21 μ (mean 25 \times 19 μ). The measurements by Gäumann (7, p. 320-322) range from 17 to 32 \times 12 to 26 μ (mostly 21 to 27 \times 16 to 22 μ) and those by Berlese, as quoted by Gäumann (7, p. 320-322) 15 to 24 \times 13 to 18 (mostly 23.5 \times 17 μ). These dimensions indicate that *P. hyoscyami* is larger than *P. tabacina*.

Peronospora nicotianae has oogonia of the type that belong in the section Calothecae, and it is, therefore, plainly specifically distinct from *P. hyoscyami*.

In 1933 Adam (1) made a comparative study of *Peronospora hyoscyami*, *P. nicotianae*, and the organism causing downy mildew of tobacco in Australia, and decided that the latter, although quite similar to *P. hyoscyami*, should be given a new name, *P. tabacina*. An important factor upon which the separation was based was the inability of the fungus from tobacco to parasitize *Hyoscyamus niger*. When this report of Adam appeared, our previous report (19) was in press. In it we pointed out that the downy mildew fungus of tobacco in the southeastern United States does not parasitize *H. niger* and, therefore, is not identical with *P. hyoscyami*. We concluded that it is either identical with *P. nicotianae* or else is an undescribed species. In view of the fact that type specimens of *P. nicotianae* were not available to us at that time, we preferred to regard it as *P. nicotianae*.

Recently, Clayton and Stevenson (5) determined, on morphologic grounds, that the tobacco downy mildew fungus of the United States resembles *Peronospora tabacina* and recommended that this name for it be used. Comparative morphological data, compiled from the accounts of all who have been concerned with studies on the two mildews, *P. nicotianae*, and *P. tabacina*, are assembled in table 2. These data show the present status of this matter.

Any one who examines *Peronospora nicotianae* and *P. tabacina* would most certainly decide that they are specifically distinct, because the

³ From correspondence.

TABLE 2.—Comparative measurements of two downy mildew fungi on tobacco

Authority	Organism	Sporangia		Oogonia		Oospores	
		Length	Width	Mean	Range	Mean	Range
Spogazzini (17)	Peronospora nicotianae	18-20 18-28	9-11 14-19	22.45 × 16.65 (a) 17.44 × 16.72	80-100 46-85 62-78	60.4- 62.3 (b)	50-80 37-70.5
Ashby ^a	"						48.1- 49.8 (b)
Clayton and Stevenson (5)	"						
Adam (1)	Peronospora tabacina	16-29 19-35	13-19 14-21	22 × 17 28 × 17 (c)	35-60	46	28-50
Angell and Hill (2)	"						30
Wolf <i>et al.</i> (19)	"	15-28	12-18	18.4 × 15	60-85 40-74	53	45-75 24-43
Wolf <i>et al.</i>	"	10.5- 24	10.5- 21	16.46 × 12.45			32
Clayton and Stevenson (5)	"						39.49
Ashby ^b	"						33.2
Ashby ^c	"						39.7

^a Ashby, S. F., from correspondence. (a) average of 30 measurements; (b) average of 50 measurements; (c) average of 100 measurements.

^b Measurements of 60 oospores from material from Australia supplied by Adam.

^c Measurements of 50 oospores from material from North Carolina supplied by the writers.

oospores of the former are considerably larger, as shown by the measurements in table 2. The areolate oospore wall of the former and the smooth one of the latter is another striking difference. The lack of accord in measurements of *P. tabacina* shows that this organism is quite variable in size. The morphologic differences between *P. hyoscyami* and *P. tabacina*, however, are not very great, as previously indicated. Some might prefer to regard the tobacco pathogen as a variety of *P. hyoscyami*, since it is unable to produce disease in *Hyoscyamus*. Rather than assign a varietal name to the tobacco downy-mildew organism, it seems preferable, as Adam (1) has done, to assign to it a name of specific rank.

OBSERVATIONS BEARING ON CONTROL

Downy mildew of tobacco has been present in North Carolina for 5 years. It has been more destructive in some seasons than in others, but there is no evidence that it is becoming less aggressive or that it may be expected to disappear in the immediate future. On the contrary, all of the evidence in hand indicates that it will remain here and will ravage tobacco seed beds year after year. During the past spring the disease appeared on large plants in eastern North Carolina and South Carolina. This indicates that this form of the disease, reported in Georgia in 1931, and prevalent in Australia every year, may become a menace in the flue-cured areas.

Growers generally have less concern about its presence in seed beds than when the disease first appeared. They expect the disease to decimate the stands of seedlings and, consequently, they sow seed beds whose area is several times as large as would be necessary if downy mildew were absent. This increases the cost of producing tobacco for each grower. In addition, the disease makes it impossible to take advantage of propitious seasons for transplanting, and is conducive to uneven stands. Tobacco, planted either too early or too late, does not yield best quality. Both factors cause loss in revenue.

If new bed sites were universally employed each year or if there were means of adequately sterilizing the old ones, it seems highly probable that downy mildew might be entirely eradicated from the flue-cured areas. Our investigations show conclusively that the employment of sites occupied by seed beds during the previous year, constitutes the most important source of primary infections. Whether growers avoid or use old bed sites, however, is a matter of personal choice. Palliative rather than preventive measures, therefore, are most likely to be adopted by them.

There is a relationship between the age of tobacco seedlings and their chances of surviving an attack of downy mildew. The mortality in a

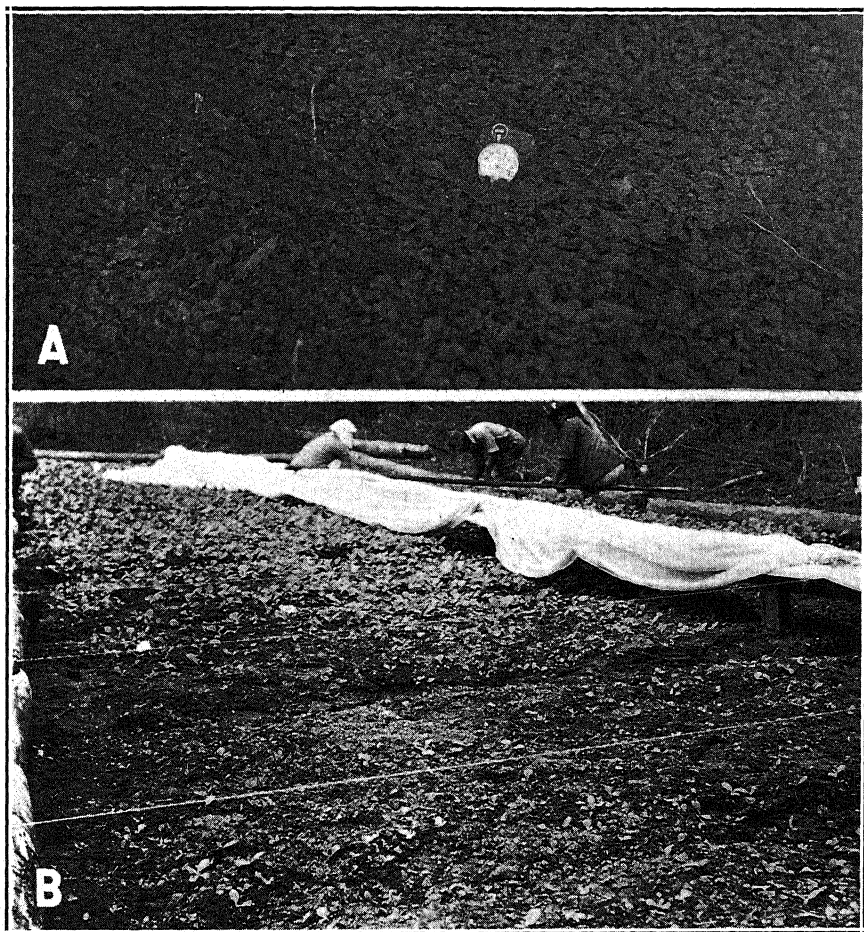


FIG. 5. A. The bare area to the left and slightly in front of the watch marks the locus of a primary infection. The seedlings were very small when photographed. B. Primarily infected seed bed near Nichols, S. C. Photographed March 28, 1935. Infection was first noted 10 days earlier. The downy mildew organism had destroyed nearly all of the seedlings in the bare area in the foreground.

primarily infected bed is always high. Such a condition is typified in figure 5. If the seedlings remain disease-free until large enough to transplant (Fig. 6), there is much less likelihood of serious losses; especially, if proper care and management of the seed beds have been provided.

Several thousand seed beds have come under our observation during the past 4 years. Evidence from these observations indicates that the location of the seed beds is an important factor in reducing the ravages of downy

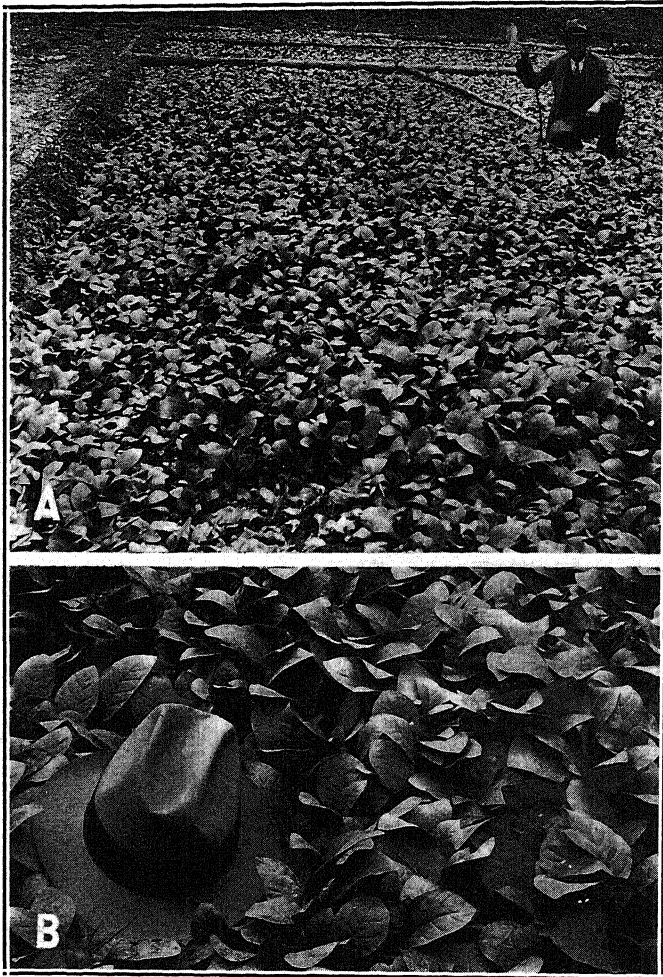


FIG. 6. A. Seed bed sown on a new site near Durham, N. C. Photographed April 29, 1935, the day after infection was first evident on seedlings in the darkened area near the corner in the left foreground. B. Close view of a portion of the bed shown in A. Photographed April 29, 1935. The seedlings were sufficiently large for transplanting. Compare the size of seedlings when primary outbreak occurred (Fig. 5A) with those that were secondarily infected.

mildew. Beds should be so located as to expose the plants to the sunshine all day. It has previously been stated (19) that more severe injury from downy mildews occurs in beds shaded all or a part of the day than in those distant from the woods. The beneficial effect of sunlight cannot be over-emphasized, since seedlings in heavily shaded beds may be completely

destroyed (Fig. 8, B), whereas beds located in a clearing several rods away, although infected, may produce a satisfactory crop of seedlings.

It is common knowledge that seedlings grow more slowly under thin seed-bed covers than under thick ones, and that those under the latter type are more tender and, therefore, are subject to more serious damage from downy mildew. Observational evidence indicates that the seed-bed covers should be removed about 3 weeks instead of a few days only, prior to the time for transplanting, for, by thus exposing the seedlings to direct sunlight they become hardened. This is an excellent procedure to use when repeated applications of nitrate of soda are made. The hardening effect of direct sunlight offsets the tender condition that normally results from the application of nitrate of soda. When growth of the seedlings is retarded by exposure to sunlight the disease may interfere with transplanting only slightly, or in some cases not at all.

Applications of nitrate of soda appear to hasten the recovery of downy mildew-affected seedlings (Fig. 7). This seems to result from stimulating the formation of new secondary roots near the base of the stem. A single application of sodium nitrate at time of seeding, or after the disease has appeared, is not so effective as several light applications made at intervals. The first application should be made in advance of an outbreak.

In the case of beds on old sites, primary outbreaks may be expected to occur at the time of the flowering of dogwood. All such beds should be given a light application of nitrate of soda approximately a week prior to this time, followed by another application as soon as the disease appears.

A few beds sown on new sites, and beds on old sites not evidencing primary infections, may be expected to develop secondary infections within 2 weeks after the appearance of primary foci in that locality. The disease will be general within another week. The producer, therefore, should make a light application of nitrate of soda on all beds, not evidencing primary infection, one week after the flowering of dogwood, followed by another application immediately after the disease is discovered in the seed bed. This procedure has proved of such value that numbers of observant growers have come to regard "soda (nitrate of soda) and sunshine" as the only means of reducing the damage from downy mildew and of hastening recovery from the disease.

Use has been made of various sprays and dusts as protectants. The difficulty involved in keeping the entire leaf surface adequately covered with fungicides indicates that there is little basis for hope that control will result from the use of such substances. Our limited experience shows that some of them afforded some measure of protection but that they are of little practical value. Those fungicides that produce any appreciable

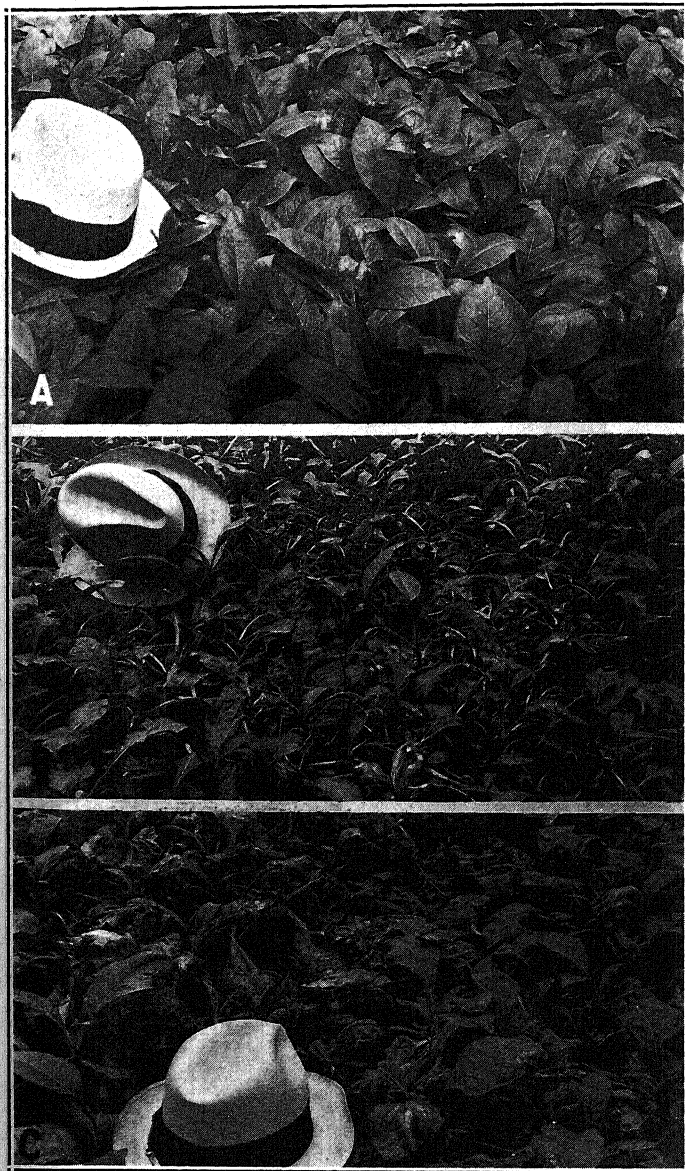


FIG. 7. Photographs taken on the same day of portions of three seed beds situated near one another. A. Nitrate of soda was applied several days prior to the outbreak of downy mildew and a second application was made while the seedlings were still severely involved. At the time photograph was taken the seedlings had recovered sufficiently to be transplanted. B. and C. No nitrate of soda applied. B. Shaded by trees during a portion of the day; the seedlings that survived had begun to recover. C. Shaded during the greater part of the day; the seedlings were severely collapsed and none were used for transplanting.

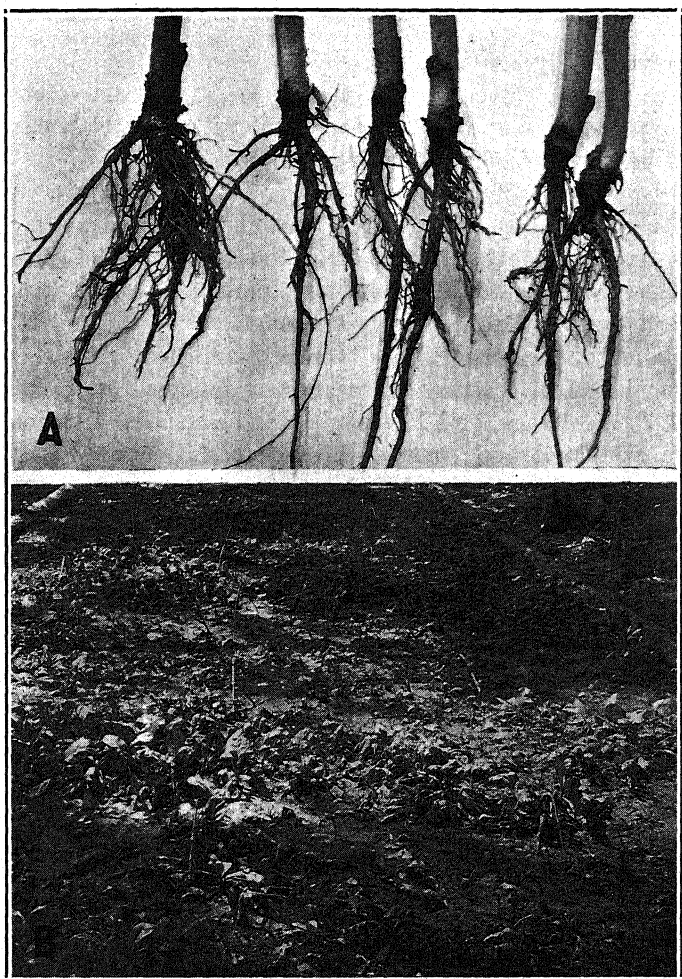


FIG. 8. A. Root systems of six tobacco seedlings. The one at the left was normal. The crowns of the remainder were discolored and they lacked secondary roots. This condition had resulted from applications of salicylanilides and naphthols as sprays. B. Tobacco seed bed closely surrounded by trees in which practically all of the seedlings were killed by the downy mildew fungus. None of the seedlings were removed for transplanting. Photographed at the close of the season for transplanting.

protection against downy mildew cause the seedlings to be stunted. Some fungicides, such as the copper salts, naphthols, and salicylanilides, interfere with the formation of secondary roots at the base of the stem (Fig. 8, A), an effect that appears to result from accumulation of the fungicide around the stems at the surface of the soil.

SUMMARY

Certain previously reported observations on downy mildew of tobacco have been confirmed. These deal with oospores as the source of inoculum for primary infections in the Carolinas, and with seed beds sown on the sites of old beds as foci of primary infection.

Primary outbreaks of the disease in any locality always occur in seed beds that occupy the sites of old beds. Secondary outbreaks are of general prevalence approximately 3 weeks after the occurrence of primary outbreaks. The general distribution of the pathogen within a locality is accomplished by means of air-borne sporangia.

Germination of the oospores has been accomplished.

The morphological features of the downy mildew fungi, *Peronospora hyoscyami*, *P. nicotianae*, and *P. tabacina*, as described by various investigators, are compared and contrasted. The pathogen on tobacco in the southeastern United States is regarded as the latter species.

It is recommended that seed beds should never be placed on old sites. This is of primary importance as a preventive measure. The most important palliative measures include the greater exposure of the seedlings to direct sunlight than is now generally employed, and the judicious administration of several applications of nitrate of soda.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA.

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SEPARATION AND ANALYSIS OF VIRUS STRAINS BY MEANS OF PRECIPITIN TESTS¹

K. STARR CHESTER

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After a rabbit is immunized with the juice of a plant containing tobacco-mosaic virus, the blood serum contains antibodies of two categories, effective, respectively, against proteins of the healthy host plant and against the virus antigen. If such a serum is treated with an adequate amount of healthy-plant juice, the antibodies against the healthy-plant proteins are removed by precipitation leaving in the supernatant fluid antibodies specific for the tobacco-mosaic disease. Such a serum is inactive toward comparable extracts containing other viruses, such as those of cucumber mosaic, various potato mosaics, and tobacco ring spot.

It is known from the work of Jensen (10), McKinney (11), and others that many strains of tobacco-mosaic virus may be derived experimentally. Similar strains also appear in nature. They are very similar in most of their properties but may be distinguished by the symptoms produced in certain hosts. Similarly, Price has shown that cucumber-mosaic virus includes distinguishable strains (12), and Böhme (4), and others have reported strains of latent potato-mosaic virus and potato-vein-banding virus. A number of investigators have attempted to use serological methods for distinguishing the strains of a given virus, but it has been found by all that serum prepared against one strain precipitates in the presence of other strains of the same virus.

In 1934 Birkeland (3) found no significant serological difference between the viruses of spot necrosis, attenuated spot necrosis, and potato ring spot, all of which are strains of the latent-mosaic virus of potato. Gratia and Manil (8), Spooner and Bawden (13), and Bawden (1) were also unable to distinguish strains of the latent potato-mosaic virus by the precipitin reaction. Beale observed that serum prepared against any one of 3 very distinct strains of tobacco-mosaic virus reacted equally well with all 3 strains employed. The writer likewise (6, 7) was unable to differentiate 20 strains of tobacco-mosaic virus from one another. Several strains of latent potato-mosaic virus were also indistinguishable from one another by means of the precipitin and complement-fixation tests. The neutralization test with the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic

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also failed in each case to differentiate strains of the same virus (5). Thus, it was evident that the strains could not be separated serologically without some further modification of the techniques.

In all of the work reported in the literature, sera prepared against one strain were tested against a number of strains in an attempt to detect quantitative differences in the strength of the reactions. It was thought, for example, that serum of an animal immunized with ordinary green tobacco-mosaic virus might precipitate juice from this strain more strongly than it would precipitate juice of a masked strain of the same virus, and vice versa. A source of error in such experiments lies in the fact that the amount of virus antigen in the various extracts may differ greatly, and the amount of antibody in two sera also may differ. Hence, quantitative differences in reactions might not be significant. In the writer's precipitin tests mentioned above, attempts were made to control this source of error by adjusting all sera to the same antibody content and by adjusting all extracts to the same virus content as determined by infection tests. But, even after taking these precautions, significant differences in the strains were not obtained. It was still thought, however, that a modified precipitin technique might reveal differences between strains of the same virus. The present paper reports the differentiation of certain strains of tobacco-mosaic virus and of certain strains of latent potato-mosaic virus by means of an absorption technique, such as was suggested by Beale in 1934 (2). The experiments herein reported were carried out with 10 strains of tobacco-mosaic virus and 3 strains of latent potato-mosaic virus. The 13 strains may be briefly characterized as follows:

1. *Tobacco mosaic* (Johnson, tobacco virus 1). Ordinary green mottling type. Caused systemic mottling in *Nicotiana sylvestris* Spegaz. and Comes.
2. *Tomato aucuba mosaic*. Yellow mottling type. Caused local necrotic spots, but no systemic infection in *N. sylvestris*.
3. *Masked tobacco mosaic* (Holmes) (9). Was systemic, but symptomless in *N. sylvestris*.
- 4-10. *Seven yellow tobacco-mosaic strains* isolated from field-type tobacco mosaic by Jensen (10) and designated here as J-102, J-108, J-201, J-202, J-302, J-303, and J-306. All caused yellow mottling in Turkish tobacco, and were distinguishable from one another in this host.
11. *Potato mottle*. A strain of latent potato mosaic that caused more or less ring-spot necrosis in tobacco, severe systemic necrosis in pepper, and moderate yellowish mottling without necrosis in *Datura tatula* L.
12. *Potato ring spot*. A strain of latent potato mosaic that caused severe ring-spot necrosis in tobacco, severe systemic necrosis in pepper, and severe mottling and systemic necrosis in *D. tatula*.

13. *Masked potato mottle*. A strain of latent potato mosaic that was isolated by the writer from healthy appearing potatoes. It caused systemic infection but no symptoms in tobacco and *D. tatula*. It caused a severe systemic necrosis in pepper.

These 13 viruses were propagated in Turkish tobacco. The tobacco juices were expressed, frozen, melted, centrifuged, and then used for inoculation of animals and for absorption and testing. Immune sera were obtained from rabbits, each of which was inoculated intraperitoneally 5 times at 3- to 4-day intervals with 6 cc. doses of juice containing the virus of one of the following diseases: tobacco mosaic, tobacco aucuba mosaic, masked tobacco mosaic, potato ring spot, potato mottle, masked potato mottle. Except for the absorption described below, all of the processes of the serological work were carried out according to the technique previously described (7).

To samples of serum of an animal immunized from one of the tobacco-mosaic virus strains were added progressively increasing amounts of tobacco juice containing a different strain of this virus. An analogous series of mixtures was prepared for the potato-virus strains. The series of mixtures was incubated for 2 hrs. at 37° C. and then for 16-20 hrs. at 5° C. The tubes were then centrifuged, the supernatant fluids were tested for precipitating activity against the virus extract used for absorbing, and that mixture was chosen for subsequent testing that contained the least amount of absorbent and yet did not react with the absorbent. In performing the precipitin tests subsequent to absorption, it was found desirable to use the absorbed sera without further dilution, while the test extracts were diluted 1:6, since preliminary tests had shown that this dilution gave the most satisfactory results.

Serum of animals immunized from each of 3 strains of tobacco-mosaic virus was absorbed with juices containing the viruses of the 2 other strains. These 6 absorbed sera were then tested against juices containing the viruses of the 10 tobacco-mosaic strains. The same procedure was followed with the viruses of the latent-mosaic strains. Each type of experiment was repeated 5 times with essentially the same results. The outcome of the tests is indicated in the following tables.

From table 1 it is seen that, after tobacco-mosaic-immune serum is allowed to react fully with aucuba-mosaic extract, the serum is still capable of reacting with tobacco-mosaic extract (test 1). The absorption of tobacco-mosaic serum by an excess of aucuba-mosaic juice does not remove all of the antibodies against tobacco mosaic. In other words, tobacco-mosaic virus extracts contain antigenic material that is lacking in aucuba-mosaic extracts. Similarly, the tests with aucuba-mosaic serum indicate that aucuba-mosaic extracts contain antigenic material that is absent from tobacco-

TABLE 1.—*Precipitin absorption tests for differentiating strains of tobacco-mosaic virus*

Serum im- mune from:	Absorbed with extract of:	No. vol- umes ab- sorbent required per vol- ume of serum	Absorp- tion pre- cipitate	Residual precipitin reactions when tested against extract of:								
				Tobacco mosaic	Aucuba mosaic	Masked tobacco mosaic	J-102	J-108	J-201	J-202	J-302	J-303
1 Tobacco mosaic	Aucuba mosaic	3	++++ ^a	++	0	++	+	0	+	0	0	+
2 Tobacco mosaic	Masked tobacco mosaic	2	+++	0	0	0	0	0	0	0	0	0
3 Aucuba mosaic	Masked tobacco mosaic	$\frac{1}{3}$	++	0	+	0	0	+	+	+	+	0
4 Aucuba mosaic	Tobacco mosaic	$\frac{1}{4}$	++	0	+	0	0	+	0	+	+	0
5 Masked tobacco mosaic	Aucuba mosaic	2	+++	+++	0	++++	+	(+)	+	(+)	0	0
6 Masked mosaic tobacco	Tobacco mosaic	$\frac{1}{2}$	+++	0	0	0	0	0	0	0	0	0

^a ++++ = very strong reaction.

+++ = strong reaction.

++ = moderate reaction.

+ = reaction safely positive but not strong.

(+) = reaction weak and questionable.

TABLE 2.—*Precipitin absorption tests for differentiating strains of potato latent-mosaic virus*

Serum immune from:	Absorbed with extract of:	No. volumes absorbent required per vol. of serum	Absorption precipitate	Residual precipitin reaction when tested against extract of:		
				Potato ring spot	Potato mottle	Masked potato mottle
7 Potato ring spot	Masked potato mottle	5	++ ^a	(+)	0	0
8 Potato ring spot	Potato mottle	3	+++	(+)	0	(+)
9 Potato mottle	Masked potato mottle	8	++	0	+	0
10 Potato mottle	Potato ring spot	6	++	0	+	+
11 Masked potato mottle	Potato mottle	3	++	(+)	0	(+)
12 Masked potato mottle	Potato ring spot	4	++	0	(+)	(+)

^a See table 1.

mosaic extracts (test 4). The absorption precipitates show, however, that the extracts of these two viruses also contain common antigenic material. The antigen constitution of tobacco mosaic might be expressed as *xy*, that of aucuba mosaic as *xz*, *x* being the common antigenic fraction, *y* the fraction peculiar to tobacco mosaic, and *z* the fraction peculiar to aucuba mosaic. Extract of the masked tobacco-mosaic strain completely absorbed tobacco-mosaic serum and serum for the masked strain was absorbed completely by tobacco-mosaic extract, *i.e.*, the masked strain has the same antigenic constitution as the green-mottling tobacco mosaic (*xy*), insofar as can be determined from these tests. At times a slight serological difference was seen between tobacco mosaic and masked tobacco mosaic, but this difference was neither strong nor constant. The tests indicate that the masked tobacco-mosaic strain is serologically very similar to the green-mottling tobacco mosaic from which it was derived, while aucuba mosaic differs from both in lacking antigens that are present in the other strains and in possessing antigens absent from the other strains.

It was felt desirable, once the antigenic differences between tobacco mosaic and aucuba mosaic were observed, to determine whether the yellow tobacco-mosaic strains, isolated experimentally from green tobacco mosaic

by Jensen (10), show any serological relationship to the naturally occurring yellow strain, aucuba mosaic. Table 1 gives the results when 7 of these experimentally-isolated strains were tested against the sera that differentiated tobacco mosaic and aucuba mosaic. Inspection of the table shows that 4 of the strains (strains J-108, J-201, J-302, J-303) contain the antigenic fraction peculiar to aucuba mosaic (z), and that one of them (J-201) contains in addition the antigenic fraction peculiar to tobacco mosaic and masked tobacco mosaic (y). The remaining strains (J-102, J-202, J-306) show the antigenic fraction peculiar to tobacco mosaic (y), but lack that peculiar to aucuba mosaic.

The tests with potato-mottle serum reveal a somewhat different relationship among the latent-mosaic virus strains. Mottle contains antigenic material that is not present in potato ring spot, but is present in masked potato mottle (test 10). Mottle also contains antigenic material that is absent from both ring spot and the masked virus (test 9). The 2 tests indicate that the 3 latent-mosaic strains under consideration are all serologically different. It is rather difficult to determine with definiteness the interrelations of potato ring spot and masked potato mottle, as the absorption reactions with these viruses are weak and difficult to read. However, a provisional antigenic analysis for these 3 strains based on reactions reported in table 2 suggests the following formulae: potato mottle, abc; masked potato mottle, abd; potato ring spot, ade.

In the analysis of the experiments, no mention has been made of the antigens present in diseased tobacco plants, but also present in healthy plants. This antigenic fraction, which may be designated as "p," is present in all 13 types of extracts and has been removed in all cases by absorption. The complete serological formulae, so far as present understanding permits their resolution, is then:

Tobacco mosaic:	pxy	Potato mottle:	pabc
Aucuba mosaic:	pxz	Masked potato mottle:	pabd
Masked tobacco mosaic:	pxy	Potato ring spot:	pade
J-102:	pxy		
J-108:	pxz		
J-201:	pxyz		
J-202:	pxy		
J-302:	pxz		
J-303:	pxz		
J-306:	pxy		

Previous precipitin, complement-fixation, and neutralization experiments have shown that tobacco mosaic, masked tobacco mosaic, and tobacco aucuba mosaic are all caused by strains of the same virus, as contrasted with

such distinct viruses as cucumber mosaic, tobacco ring spot, etc. By the use of absorption tests, tobacco mosaic and masked tobacco mosaic are found to be very similar serologically, while aucuba mosaic differs significantly from both. One might think that aucuba mosaic differs serologically from ordinary tobacco mosaic because aucuba mosaic is a natural variant, which has existed in nature apart from tobacco mosaic for some time. Yet the tests show that some of Jensen's yellow strains possess the antigenic peculiarities that distinguish aucuba mosaic from tobacco mosaic. The serological evidence indicates that aucuba mosaic is as closely related to tobacco mosaic as are a number of the strains isolated experimentally from tobacco mosaic by Jensen.

From the evidence reported above, it is concluded that the absorption technique used permits the differentiation of strains of the same virus type. By using such a technique it has been shown that serological differences exist among certain strains of tobacco-mosaic virus. Strains of latent potato-mosaic virus were also separable from one another serologically. It was found that the precipitin absorption technique described, not only serves to distinguish virus strains, but also gives some index of the constitution of the different viruses.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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PATHOGENICITY TESTS WITH BOTRYTIS SPP. WHEN INOCULATED INTO APPLES¹

OTTO F. SCHNELLHARDT² AND F. D. HEALD³

(Accepted for publication October 21, 1935)

INTRODUCTION

Oudemans (7, v. 3: 495) listed 3 species of *Botrytis* as occurring on apple fruit. The first of these *Botrytis ramosa* Pers., is imperfectly described in Saccardo, but it is probably synonymous with *B. cinerea*. The other 2 species, *B. vera* Tr. and *B. vulgaris* Fr. are considered by Lindau (5, p. 284-285) to be synonyms for *B. cinerea* Pers. A new species, *Botrytis mali* Ruehle, (9) has been isolated from apples in Washington. According to Whetzel,⁴ the binomial *Botrytis cinerea* has no sound taxonomic significance, and, until the taxonomy of this fungus can be established, the writers prefer using "*Botrytis* spp. of the cinerea type." From the evidence obtained in the pathogenicity tests and the isolations from decayed fruit in Washington it appears that *Botrytis* spp. of the cinerea type are the only species of frequent occurrence on apples in storage in this state.

Previous work (10) indicates that gray mold may become destructive as a rot of stored apples in Washington wherever there is considerable rain during the harvesting period. In certain lots of apples examined in the Wenatchee district in 1933-34, gray mold caused a greater percentage of decay than blue mold. Inoculation experiments have shown the gray mold organism to advance more rapidly on apples in cold storage than blue mold (Fig. 1). The spot-rot symptom, evident in fig. 1, A, was due to a temperature relationship, as described by Heald and Sprague, (2) and developed after the apples were removed from cold storage.

Bach⁵ and Ruehle⁶ found different forms of *Botrytis* of the cinerea type exhibiting little difference in pathogenicity. Huber⁷ designated two strains

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² Formerly Research Assistant in Plant Pathology, Washington Agricultural Experiment Station, Pullman, Washington.

³ The writers acknowledge their indebtedness to the workers listed in table 1 who contributed isolations or diseased specimens for inoculation work.

⁴ From letter dated November 2, 1934.

⁵ Bach, W. J. The rots of apples in Eastern Washington. 45 p. 1922. (Unpub. thesis. State College of Washington Library.)

⁶ Ruehle, G. D. Fungi which cause decay of apples in cold storage. 163 p. 1930. (Unpub. thesis. State College of Washington Library.)

⁷ Huber, G. A. The fungous flora of the normal apple: I. The sources of contamination and spore load. II. Fungi present and their relation to decay. 103 p. 1931. (Unpub. thesis. State College of Washington Library.)

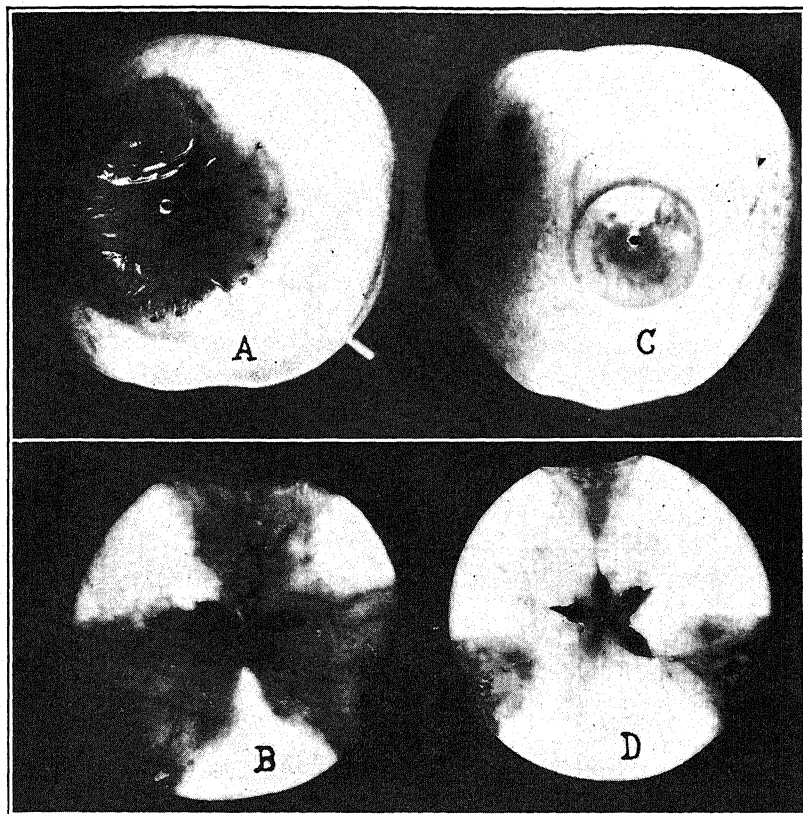


FIG. 1. Gray-mold and blue-mold lesions on Rome Beauty apples. Fruits artificially inoculated and held at 0° C. for 36 days. A and B. Gray mold (*Botrytis cinerea*). C and D. Blue mold (*Penicillium expansum*).

of the *Botrytis cinerea* type as No. 1 and No. 2 with the former producing slightly more rapid decay than the latter. Ruehle^s found that *Botrytis alli*, isolated from an onion bulb affected with neck rot, was capable of producing a fairly rapid decay of apples when these were punctured and inoculated with spores. The onion Botrytis was not so virulent on apples as either *Botrytis cinerea* or *Botrytis mali*, and produced lesions much more slowly at the various temperatures.

MATERIALS AND METHODS

For this study various isolations of *Botrytis* spp., mostly of the cinerea type, as shown in table 1, were secured and inoculated into apples and the rate of decay recorded.

^s Loc. cit.

TABLE 1.—*List of the Botrytis isolations used for inoculations into apples.*

No.	Species	Host	Locality	Worker
B-1	<i>B. cinerea</i> type	Apple fruit	Pullman, Wn.	George D. Ruelhle
B-2	"	"	Massachusetts	H. H. Whetzel
B-3	"	Pea pods	Montesano, Wn.	Self
B-4	"	Dogwood twigs	Seattle, Wn.	G. A. Huber
B-5	"	Blueberry twigs	Puyallup, Wn.	"
B-6	"	Geranium shoots	Spokane, Wn.	Self
B-7	"	Gloxinia shoots	Spokane, Wn.	"
B-8	"	Pear fruits	Yakima, Wn.	Harley English
B-9	"	"	Yakima, Wn.	"
B-10	"	"	Yakima, Wn.	"
B-11 ^a	"	Apple ?	England	Wm. Brown
B-12	<i>B. cinerea</i> ^b	Crassula perforata	England	W. B. Brierley
B-13	<i>B. cinerea</i> lini	Flax	The Netherlands	V. Beyma
B-14	<i>B. trifolii</i>	Clover	The Netherlands	"
B-15	<i>B. fabae</i>	Horse bean	Spain	J. R. Sardina
B-16	<i>B. cinerea</i> type	Feijoa	Riverside, Cal.	W. T. Horne
B-17	"	Guava	Riverside, Cal.	"
B-18	<i>B. tulipae</i>	Tulip	Pullman, Wn.	Earl Anderson
B-19	<i>B. cinerea</i> type	Apple fruit	Lavington, B. C.	G. E. Wooliams

^a Cultures 11-15 were obtained from the Centraal Bureau voor Schimmellecultures, Baarn, The Netherlands.^b Strain producing albino sclerotia.

Single-spore isolation cultures of these *Botrytis* species, with the exception of B-12 and B-13, were made according to Keitt's method (4) and used for inoculation work. Since no fructification was obtained in cultures B-12 and B-13, mycelium was used as inoculum.

Huber's method (3) being used, 15 prime maturity Jonathans were inoculated with each *Botrytis* culture and held at 3 different temperatures for the periods stated (Table 2). The inoculated apples were wrapped in sterile oiled wraps, and precautions were taken to prevent mixing the species of *Botrytis* and to exclude any outside contamination. Reisolations were made from the developing lesions and in each case the fungus used for inoculum was recovered and its identity verified by comparison with the original cultures.

RESULTS OF INOCULATIONS

The results of the pathogenicity tests indicate that *Botrytis* spp. of the cinerea type caused decay of apples at a fairly uniform rate, regardless of the host from which they were isolated. Recent isolations of *Botrytis* spp. seemed to show a more rapid advance of decay than those that had been cultured for some time. This is evident from the figures in table 2. The cultures B-1, B-12, B-13, B-14, and B-15 had been grown on artificial media some time before the inoculations were made. Three species of *Botrytis*, other than the cinerea type *i.e.*, B-14, B-15, and B-18, were only weakly parasitic. The data presented show an increased rate of decay as the temperature is raised and the storage period lengthened (Table 2).

The decay produced by the *Botrytis* isolation from *Crassula perforata* (Table 1) was a small, sunken lesion; the horse-bean isolation produced a larger, somewhat raised, smooth lesion, while the *Gloxinia* isolation showed a firmer, still larger lesion (Fig. 2, D, to F). The surface views of lesions of apples inoculated with *Botrytis* species of the cinerea type are shown (Fig. 2, A to C). Cross sections of inoculated apples showed that the fungi were fairly uniform in their rate of decay, except in B-12 and B-15, while in those two decay was slower, which is also shown by the surface of the lesions (Fig. 2, D, E). The advance of the fungi B-15, B-12, B-11 and B-7 are shown in cross section in fig. 3.

The isolation of *Botrytis* from *Gloxinia* always produced a light red pigment in the culture media and this characteristic was retained throughout the experiments. This cultural character of strains of *Botrytis cinerea* has been recognized also by Heald and Sprague (2), while Peyronel (8) in France, working with isolations from 150 hosts, obtained 12 different strains of *Botrytis* (of the *B. cinerea* type) that produced an abundant red pigment.

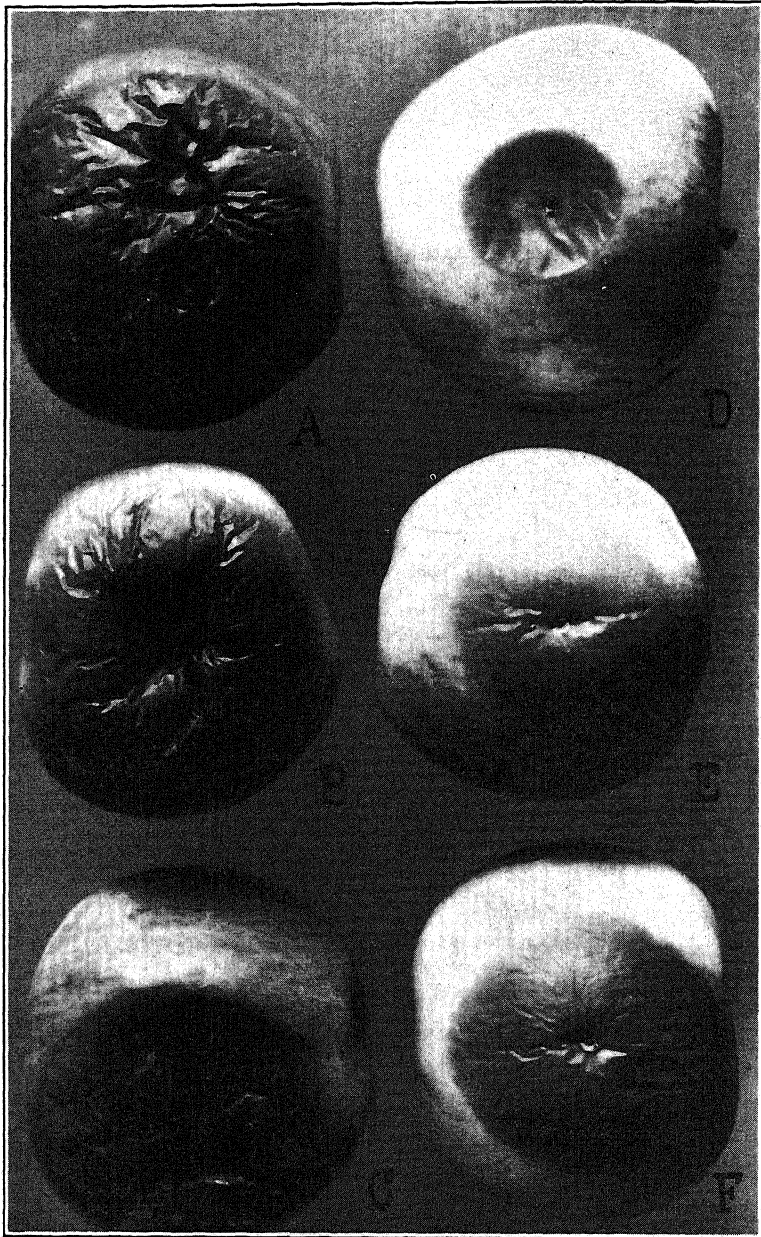


FIG. 2. *Botrytis* spp. inoculations showing diameter of lesions on surface of apples after 30 days at cold storage. A. B-11. B. B-2. C. B-1. D. B-12. E. B-15. F. B-7.

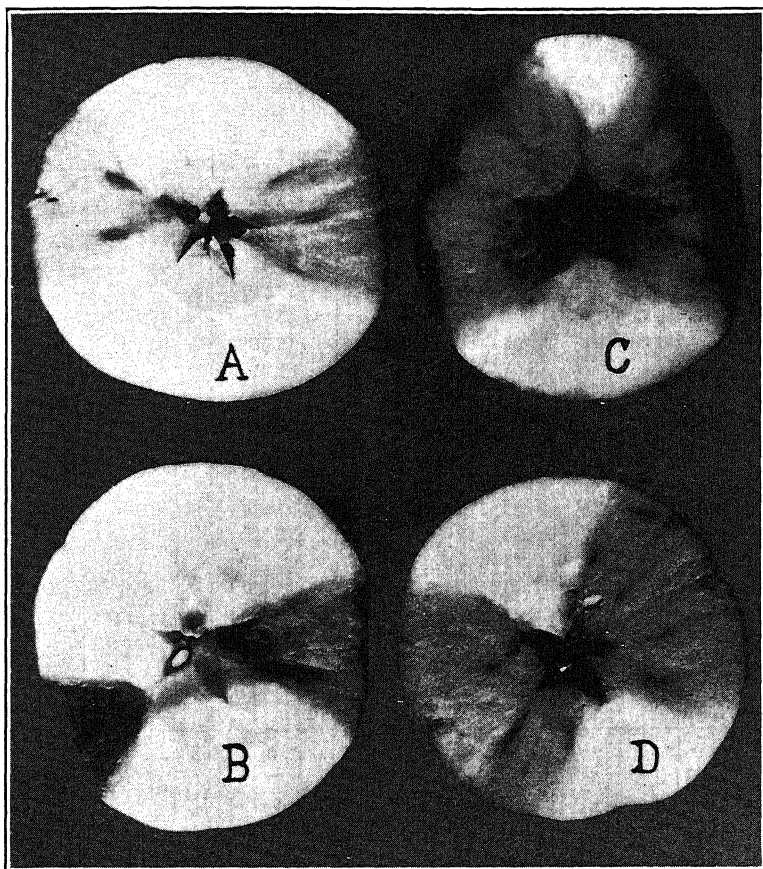


FIG. 3. Cross sections of apples inoculated with various *Botrytis* spp. showing advance of lesions in apple tissue. A. B-15. B. B-12. C. B-11. D. B-7.

More extensive inoculation experiments were carried on with B-1, the form previously isolated by Ruehle⁹ from apples. Punctured apples of 3 varieties were dipped in a heavy suspension of spores and held in cold storage for 30- and 60-day periods (Table 3).

Two apples in the Jonathan variety and 2 in the Rome Beauty were entirely decayed at the end of the 60-day period in cold storage. These results were similar to those reported by Heald and Ruehle (1) when ripe Jonathans were one-half to three-fourths decayed at the end of a 60-day incubation period at 0° C. It seems the pathogenicity of this *Botrytis* sp. was unaffected by continued culture on artificial media.

⁹ *Loc. cit.*

TABLE 2.—*Diameter in millimeters of lesions produced on sound, ripe Jonathan apples by various Botrytis spp.*

Culture Number	Room temperature (72–74° F.)		Common storage (40–40° F.)		Cold storage (32–35° F.)	
	6 days	14 days	6 days	14 days	30 days	44 days
B- 1	22.23	50.50 ^{bb}	5.66	10.77	30.78	42.04
B- 2	28.65	58.22 ^{cc}	8.00	17.96	44.93	64.84
B- 3	36.72	aa	8.87	19.47	52.60	71.29
B- 4	37.04	aa	8.00	20.20	53.93	73.30
B- 5	36.81	aa	47.63	65.72
B- 6	26.28	64.86	6.60	17.78	50.00	73.65
B- 7	34.80	aa	7.28	12.21	39.53	51.34
B- 8	28.94	aa	9.07	18.61	47.70	65.12
B- 9	24.14	71.16	8.08	17.66	47.06	63.91
B-10	36.85	aa	6.20	16.26	50.03	65.23
B-11	17.57	39.57	4.33	9.25	46.36	69.83
B-12 ^s	19.00	30.66	5.66	7.06	17.14	27.54
B-13 ^s	2.47 ^a	46.50	.89 ^e	2.25 ^f
B-1400	3.23 ^d
B-15	2.95 ^b	33.35	.00	.00	19.26	26.86
B-16	25.57	66.61
B-17	14.38	56.42
B-18	3.42 ^c	21.27
B-19	22.61	62.16

The superior letters indicate that, of the 30 punctures, a limited number developed decay as follows: a, 5; b, 7; c, 6; d, 2; e, 5; f, 11.

aa All lesions coalesced; cc Lesions on four apples coalesced.

bb Lesions on three apples coalesced; s Mycelium used for inoculum.

TABLE 3.—*Development of gray mold at cold storage temperatures (32–35° F.) when punctured apples were dipped in a spore suspension. Apples inoculated November 8, 1934*

Variety and number of apples	Average diameter of lesions in millimeters	
	30 days	60 days
Rome Beauty (28)	17.03	58.53
Jonathan (28)	12.68	33.65
Delicious (27)	28.25	53.62

DISCUSSION

Isolations of *Botrytis* spp. (mostly of the cinerea type) secured from various hosts, were used as inoculum on punctured, ripe Jonathan apples, and their pathogenicity was studied under room temperature, common storage, and cold-storage conditions. Isolations of the *Botrytis* spp. of the

cinerea type were all capable of causing rapid decay of apples, some rotting the entire apple after 14 days at room temperature. Even at the cold storage temperatures lesions of 50 mm. or more were produced at the end of the 30-day period. The *Botrytis* species from tulip, clover, and horse bean were only weakly parasitic. Two of the *Botrytis* species obtained from The Netherlands labeled and previously identified as the cinerea type, failed to sporulate and were also of little concern as parasites. The others, however, all caused a rapid decay of apples, some even greater than B-1, the original species isolated from apple by Ruehle. It appears that the *Botrytis* species of the cinerea type studied were strongly pathogenic on apple fruits, with the exception of B-12 and B-13. The strains that had been recently isolated from their hosts exhibited the greatest pathogenicity, while continued culturing on artificial media tended to decrease their virulence.

The results indicate that the presence of any of the *Botrytis* spp. (of the cinerea type) where apples are grown or packed may become a potential source of decay. Melchers (6) demonstrated that gray mold causing a blossom blight and leaf spot of geranium was the same as the one attacking head lettuce in the greenhouse. The limited data in this investigation tends to indicate that the gray mold on fruits, vegetables, and ornamental plants would be interchangeable in their pathogenicity. Where pears and apples are handled in the same packing houses, the gray mold infecting one could easily infect the other. Vegetables, such as peas with infected pods, might provide spores for dissemination to apple fruits. Ornamental plants, decayed by gray mold in the greenhouse and discarded outside, could supply sufficient inoculum to cause decay of apples that may be handled near by.

SUMMARY

1. Gray mold may become destructive as a rot of stored apples in Washington wherever there is considerable rain during the harvesting period. Gray mold advances more rapidly on apples in cold storage than blue mold.
2. Nineteen isolations of *Botrytis* spp., mostly of the cinerea type, were secured and inoculated into apples and the rate of decay recorded.
3. Species of *Botrytis* other than the cinerea type were only weakly parasitic and appear to be of little importance as a cause of apple decay.
4. *Botrytis* species of the cinerea type are strongly parasitic when inoculated into apples.
5. Recently isolated species seem to exhibit greater pathogenicity than those having been cultured on artificial media.
6. Plants affected with gray mold in the vicinity of apple orchards and packing houses are a potential source of inoculum that may result in decay of stored fruit.

DEPARTMENT OF PLANT PATHOLOGY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON.

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PHYTOPATHOLOGICAL NOTES

An Abnormal Graft Reaction in Potato Resulting from a Virus Infection of a Scion on a Resistant Stock.—In connection with studies of resistance of potato to viroses, it was found that Green Mountain scions infected with latent mosaic (X-virus) developed aerial tubers, rolling of the leaves, and marked stunting when grafted on healthy U. S. D. A. seedling number 41956 (Fig. 1, A), which is known to be resistant to latent mosaic.¹ Green Mountain seedlings, without latent mosaic, grafted on healthy 41956 (Fig. 1, B) did not develop the above abnormalities, but Green Mountain seedlings infected with latent mosaic (Fig. 1, C) reacted like the Green Mountain. This reaction has been obtained where the Green Mountain seedlings were inoculated after the grafts were made, as well as where they were infected with latent mosaic before the grafts were made. It has been obtained in all varieties, carrying the latent mosaic that have been tested. In all instances where there was a good aerial tuber formation, there was a lack of development and early necrosis of the underground parts of the stock.

A similar abnormal reaction has been observed in grafts of mild-mosaic-infected scions on Irish Cobbler stocks, which are known to be somewhat resistant to this disease. In these grafts, the rate of formation of the aerial tubers was slow as compared with that where the latent-mosaic-infected scions were grafted on seedling 41956. This suggests that the Irish Cobbler may not be so resistant to mild mosaic as is 41956 to the latent mosaic. Thus far, 41956 has not been systemically infected with latent mosaic so that it carried through the tubers. It, however, has developed a very slight necrotic spotting of the leaves, which, in some instances, was barely noticeable, when it was grafted on a plant having latent mosaic.

Results, thus far, indicate that the general principle of grafting infected scions on potatoes to determine their reaction to the virosis concerned may be of much value in studies on resistance to viroses.—W. P. RALEIGH, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Privet and Jasmine Galls Produced by a Species of Phomopsis.—

Privet.—Two hedges of the common privet, *Ligustrum vulgare* L. growing in the District of Columbia, were found badly diseased and beginning to die in patches. Examination showed that many of the stems had nodular galls near the surface of the ground; others had them on aerial parts, also. The galls ranged from 1 to 3 cm. in diameter. The largest were low down on the stems (Fig. 1, A-C). This type of gall on privet has been reported

¹ Schultz, E. S., C. F. Clark, R. Bonde, W. P. Raleigh, and F. J. Stevenson. Resistance of potato to mosaic and other virus diseases. *Phytopath.* 24: 116-132. 1934.

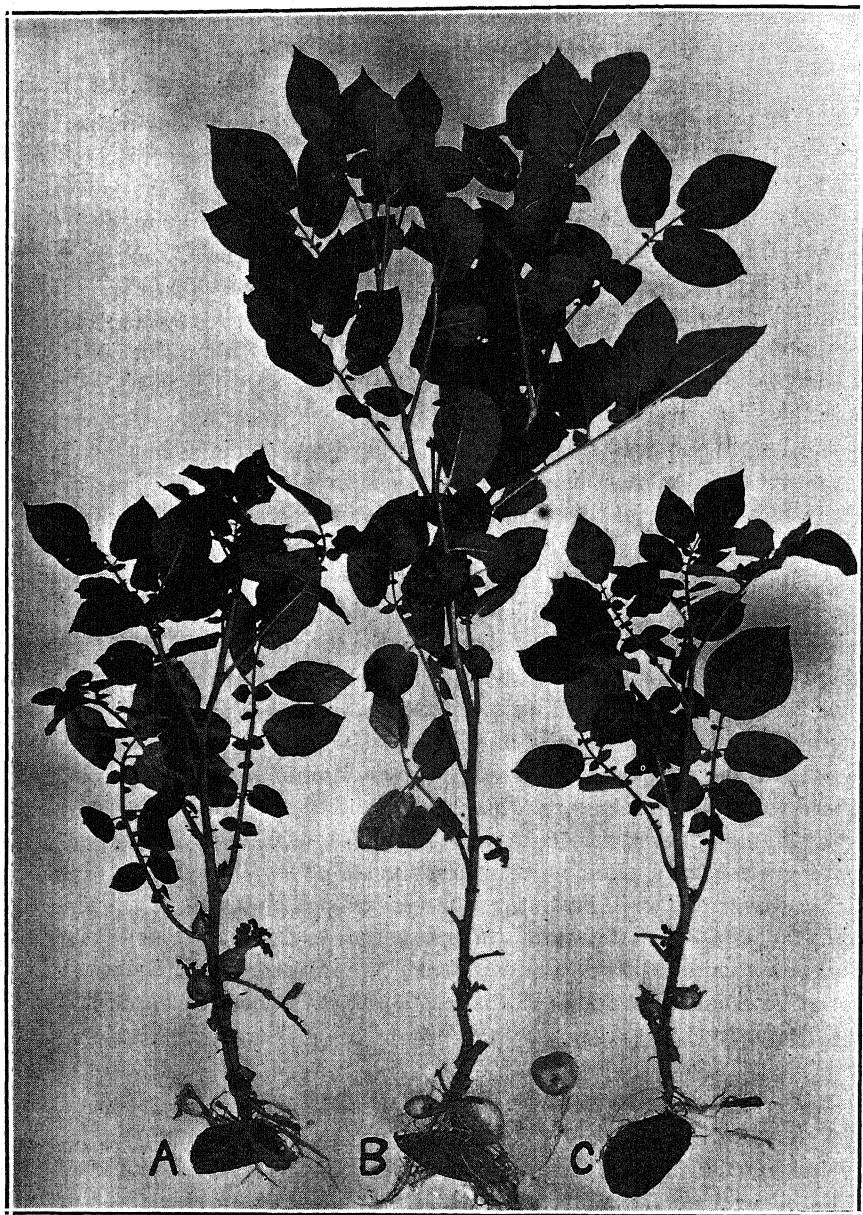


FIG. 1. Three potato stem grafts with healthy U. S. D. A. seedling number 41956 as stocks. In Graft A the scion is a Green Mountain infected with latent mosaic; in B the scion is a virus-free Green Mountain seedling; while in C the scion is a Green Mountain seedling infected with latent mosaic. Note the aerial tubers, rolling of the upper leaves, marked stunting, and lack of development of the underground parts in grafts A and C.

from several southern States, and has been called crown gall, as though it were produced by *Bacterium tumefaciens*.

On microscopic examination, bacteria were found in the outgrowths, and a yellow bacterium was isolated from 8 galls. *Bacterium tumefaciens* did not appear on the isolation plates. On some of the older galls that were studied small pycnidia containing Phoma-like spores were observed. At first these were considered of a secondary nature.

Dr. Gotthold Steiner, the nematologist, examined some of the diseased material and found the pathogenic strawberry nematode *Aphelenchoides fragariae* (Ritzema-Bos) Steiner present in small numbers. To eliminate the strawberry nematode as the possible pathogen, it was necessary to prove that a bacterial or fungous organism was the cause of the disease. Various colonies of the yellow bacterium from the different isolations were inoculated into healthy privet plants in hedges and in pots in the greenhouse, but no infection followed.

In studying microscopic sections of the galls, so little fungus mycelium could be detected that it seemed a fungus could not be the cause of the disease. However, a Phoma was isolated from some of the younger galls and later inoculated into *Ligustrum vulgare*, both in the greenhouse and out-of-doors. Good infections followed in about 50 per cent of the inoculations made out-of-doors and in over 60 per cent of those made in the greenhouse, where the moisture conditions could be controlled better. Infections, so far as the work has gone, have occurred only through wounding the tissue when the spores are brushed on. The galls produced by inoculations reached 3 cm. in diameter in 5½ months (Fig. 1, D).

The fungus has been reisolated and galls produced on *Ligustrum vulgare* with the reisolated pathogen. Another privet, *Ligustrum amurense* Carr., also has been inoculated with the fungus. Galls formed very slowly on this species, and up to the present time, 4 months after inoculating, are still small—less than 1 cm. in diameter. Olive, *Olea europaeae* Linn., and winter jasmine, *Jasminum nudiflorum* Lindl., two shrubs related to the privet, are also susceptible to the disease, small but definite galls having been produced by inoculations with the privet gall fungus.

Four different strains of *Bacterium tumefaciens*, an organism infectious to so many unrelated plant families, failed to infect privet, while the same cultures were infectious to other hosts in the same greenhouse.

In culture, the Phoma-like pycnidia are produced in great numbers. No perfect stage has yet been found on the plants on old hibernated galls or on excised galls that had overwintered on the soil. But from galls kept 10 months in the refrigerator around 12° C., then taken out and moistened, Phomopsis spores, including the scoliospore type exuded from the pycnidia. It is not yet known if this is a new species.

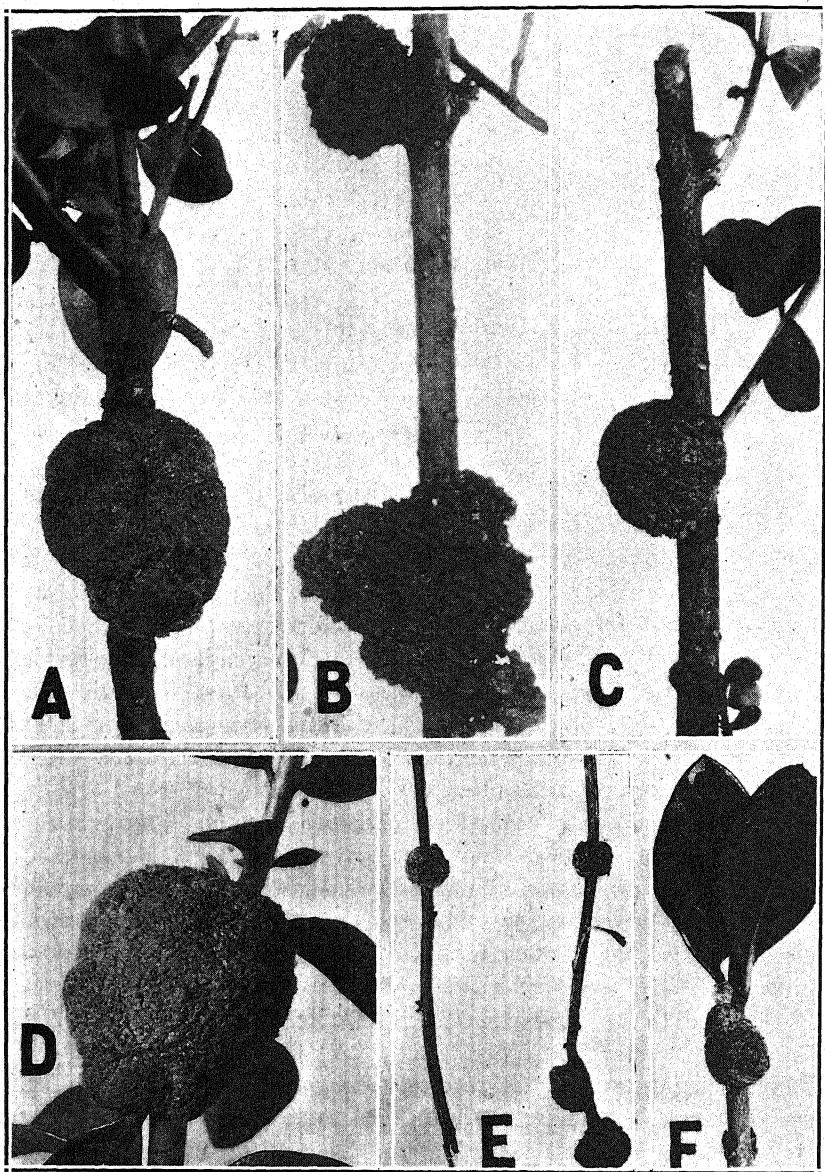


FIG. 1. A-C. Types of gall occurring on privet hedges in the District of Columbia. D. Gall produced on privet by inoculating with *Phomopsis* isolated from privet gall. Time 5½ months. E. Natural infection occurring on *Jasmine nudiflorum*. F. Jasmine gall produced on privet by inoculation with the Jasmine isolation. Time 1½ months. All natural size.

Jasmine.—Small galls of a finely nodular type occur in the South on *Jasminum nudiflorum* Lindl. They occur along the stems, as well as near the ground, and, when they do not weaken or kill the stem, make it unsightly (Fig. 1, E). The galls are usually smaller than those on the privet. What appears to be the same fungus found in privet galls was isolated from the jasmine and when inoculated into *J. nudiflorum* and *Ligustrum vulgare*, it produced galls readily, the jasmine galls on the privet becoming larger than those on its host plant from which it was isolated (Fig. 1, F). —NELLIE A. BROWN, Bureau of Plant Industry, Washington, D. C.

Morphological Aspects of Gymnosporangium Galls.—The morphology of the "cedar apples" on *Juniperus virginiana* L. caused by *Gymnosporangium juniperi-virginianae* Schw. has been studied during half a century, with contradictory results.

Sanford,¹ perhaps the first to study the galls, decided that they arose from leaf tissue. This opinion apparently was supported by Kern,² Heald,³ Reed and Crabill,⁴ and Coons.⁵ Engler and Prantl⁶ seem to agree with Wörnle⁷ whose extensive research led him to conclude that the galls are of stem origin. Stewart⁸ says that the galls arise from the axils of the leaves and evidently are transformed axillary buds.

The author has observed in extensive surveys a greater abundance of galls on awn-shape (Fig. 1, B) than on scale-like leaves (Fig. 1, A). Although the awn-shape leaves usually are considered to be the juvenile form, mature trees whose foliage is all of this type frequently are found (Fig. 1, C). The galls are abnormally abundant and appear to be attached to the stem. Careful examination, however, shows each gall as attached only to a single leaf (Fig. 1, D).

An explanation of this apparent difference in susceptibility between the two types of leaves was sought. One hundred galls taken from many trees

¹ Sanford, E. Microscopical anatomy of the common cedar apple (*Gymnosporangium macrospus*). Ann. Bot. 1: 263-268. 1888.

² Kern, F. D. A biologic and taxonomic study of the genus *Gymnosporangium*. N. Y. Bot. Gard. Bull. 7: 391-494. 1909-1911.

³ Heald, F. D. The life history of the cedar rust fungus *Gymnosporangium juniperi-virginianae* Schw. Neb. Agric. Exp. Sta. Ann. Rep. 22: 105-113. 1909.

⁴ Reed, Howard S., and G. H. Crabill. The cedar rust disease of apples caused by *Gymnosporangium juniperi-virginianae* Schw. Virginia Agricultural Experiment Station. Tech. Bull. 9. 1915.

⁵ Coons, G. H. Some investigations on the cedar rust fungus, *Gymnosporangium juniperi-virginianae*. Neb. Agr. Expt. Sta. Ann. Rep. 25: 217-245. 1912.

⁶ Engler, A. E., and K. Prantl. Die Natürlichen Pflanzenfamilien. 1: 570. 1900.

⁷ Wörnle, P. Anatomische Untersuchung der durch *Gymnosporangium*-Arten hervorgerufenen Missbildungen. Forstl. Naturw. Zeitsch. 3: 68-84, 129-172. 1894.

⁸ Stewart, Alban. An anatomical study of *Gymnosporangium* galls. Amer. Jour. Bot. 2: 402-417. 1915.

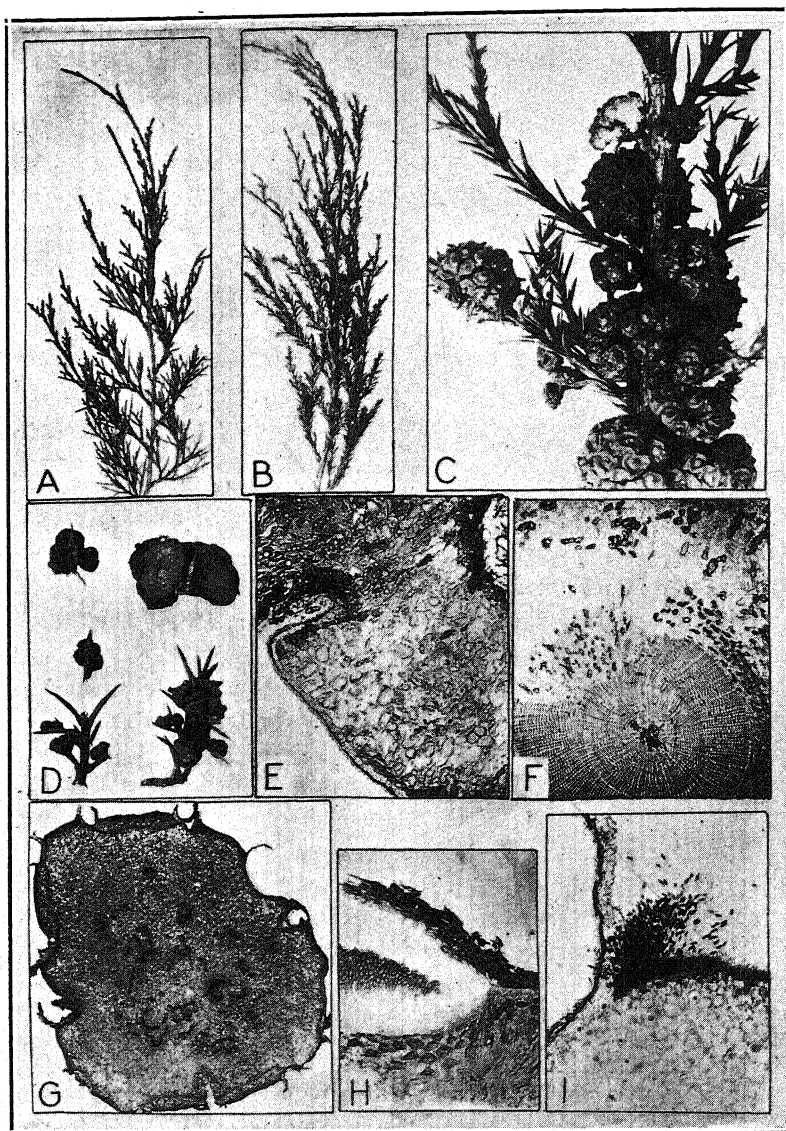


FIG. 1. A. Branch of red cedar showing the scale-like leaves or the common, mature type. B. Branch showing the awn-shape leaves, which are usually described as juvenile growth. C. Twig bearing abnormally placed galls, which are attached to the leaves of the awn-shape type. D. Galls taken from twig illustrated in "C," showing their leaf attachment. E. Photomicrograph, showing a portion of a gall which originated from an awn-type leaf. F. Photomicrograph, showing a portion of a gall attached to a stem which bore scale-like leaves. G. Thick median section of a gall showing the telial horns lifting up the cortex caps. H. A portion of a gall shortly before the absorption of water and the lifting of the cortex cap. I. Illustration of the lifted cortex cap and emergence of the spore-horn apex.

bearing the scale-like leaves and the same number from numerous trees bearing the awn-shape leaves were cut in small cubes, desilicified for 2 weeks in a 25 per cent solution of commercial hydrofluoric acid, and then prepared for sectioning by the standard celloidin process. The sections were cut $10\ \mu$ in thickness and stained with Heidenhains iron-haematoxylin. These stained preparations indicate that the galls taken from trees with awn-shape leaves are of leaf origin (Fig. 1, E) whereas those from trees with scale-like leaves are of stem origin (Fig. 1, F).

Another phenomenon upon which opinion has been divided is the method of emergence of the gelatinous spore horns through the tough cortex of the gall. According to Stevens⁹ the upper cells immediately below the surface of the cortex, where the teliospores are to form, gradually swell, become turgid, and help rupture the corky layer covering them. A technique was developed whereby clearly visible sections could be produced easily and rapidly from fresh material. Galls in various stages of development were mounted and frozen in Le Page's glue and sectioned with the freezing-microtome apparatus. The frozen glue held the gall firmly in place for sectioning, but at the same time was somewhat flexible, thus allowing thin, untorn segments to be made. Staining was not necessary to bring out the structure of the galls, as there was sufficient tannin in the fresh material. Analysis of these sections revealed circular cortex caps, lifted and pushed aside by the emerging spore horn (Fig. 1, G-I). The caps can be observed on the surface of the gall as slight pit-like depressions.—PAUL R. MILLER, Bureau of Plant Industry.

Thyrostroma compactum on *Ulmus pumila*.—Since the spring of 1934, the writer often has observed *Ulmus pumila* L. attacked by fungi in Illinois. In a number of instances such trees have died. The most prevalent type of fungus injury observed was the formation of cankers, with which various fungi were associated. In most instances these cankers occurred on the trunks of the trees near or at the soil line, but they have been observed also on main stems near the tops of 25-foot trees.

Among these cankers, the one shown in figure 1, A, is especially interesting. It occurred on the trunk of a 5-foot tree, just above the lowest branch and about 2 feet above the ground.

This tree had been under general observation along with many others, throughout the 1934 growing season and early spring of 1935, and had shown no striking symptoms of disease. On May 17, 1935, however, its upper portion, on which the leaves had partially unfolded, was found

⁹ Stevens, Edith. Cytological features of the life history of *Gymnosporangium juniperi-virginianae*. Bot. Gaz. 89: 394-400. 1930.

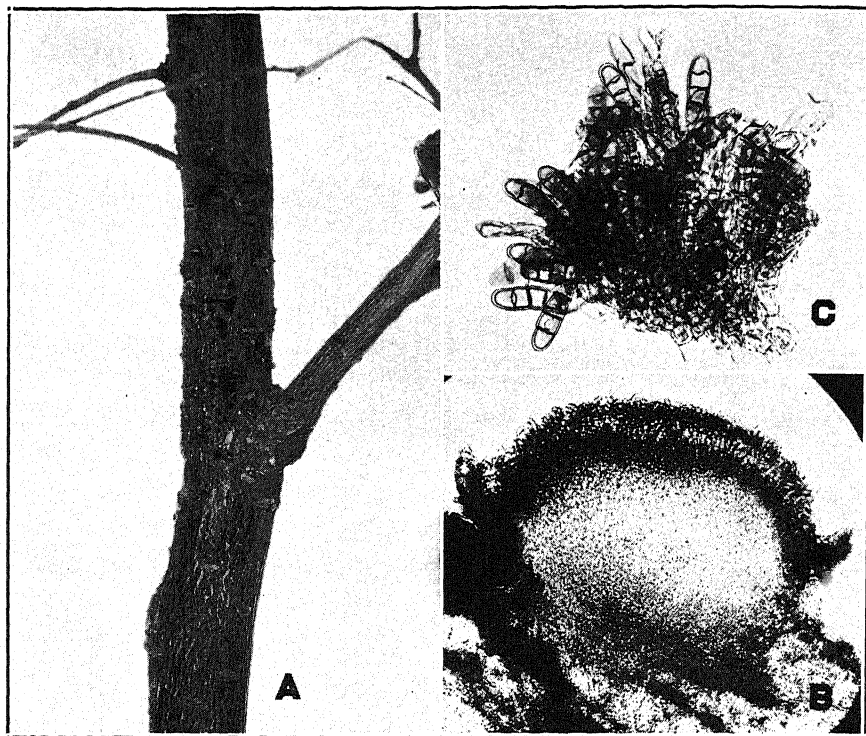


FIG. 1. *Thyrostroma compactum* on *Ulmus pumila*. A. Canker, with shrunk bark and erumpent, black tubercles. B. Tubercle in section, showing structure, relation to host tissues, and conidia on the outer surface. C. Fragment of a tubercle showing conidiophores and conidia.

wilted, the wilt involving the main stem and all lateral branches except the lowest one. Examination revealed canker.

In the cankered region the outer host tissues did not crack or slough off, but became dry, collapsed, and tightly shrunk around the stem, producing the effect of girdling either by the death of tissue or by pressure.

Some time after the appearance of the canker (3 to 4 weeks, as judged by the size of the wilted leaves) tubercles of a fungus determined as *Thyrostroma compactum* (Sacc.) v. Höhnelt appeared over most of the cankered area (Fig. 1, A). These tubercles, shown in section in figure 1, B, become erumpent at maturity. They are laid down, fundamentally, in the phellogen-phelloderm region of the stem, these tissues being disintegrated wherever a tubercle develops and being replaced by a compactly interwoven hyphal mass, the base of the tubercle. The growing tubercle ruptures the overlying phellem, epidermis, and cuticle, forcing these tissues back so as to form a collar around the erumpent mature tubercle.

Hyphae within the host continue to develop at the bases of tubercles, eventually forming below each tubercle a fairly distinct, dense, brown mass that sometimes bears cavities suggestive of developing perithecial locules. This basal structure occupies the region of the cortex, endodermis, and pericycle, and, enlarging, crushes into the more or less disintegrated outer phloem, the sclerenchymatous cells of which are considerably decomposed by the vegetative fungal hyphae. Laterally from this base, a loosely interwoven subiculum-like, web of dark hyphae develops, which replaces apparently dissolved cortex, endodermis, and pericycle.

After the tubercles have become erumpent, they appear as dark brown to black, circular, strongly convex, compact cushions protruding through the host tissue. They are 600 to 900 μ in diameter and 350 to 500 μ high.

Conidia (Fig. 1, C) are produced singly on short, tan, rod-like conidiophores over the outer face of the tubercle and accumulate as a compact mass on the top of the tubercle. They are oblong, somewhat clavate, bicellular to multicellular, muriform in part, somewhat constricted at the septa, and deep tan to brown. They range from 13.3 to 16.6 $\mu \times 39$ to 54 μ and average 14.5 $\mu \times 46.5 \mu$.

While repeated attempts to germinate conidia on potato dextrose and cornmeal agar and in sterile distilled water were unsuccessful, as were also attempts to isolate the fungus from diseased sapwood, bark, and tubercles, the relationship of the fungus to the canker is obvious.

This fungus was first described by Saccardo in 1876 under the name *Coryneum compactum*,¹ but in 1882 he transferred it to *Steganosporium*.² In 1907 von Höhnelt³ transferred it to *Thyrococcum*, and, in 1911⁴, reasigned it to his genus *Thyrostroma*, of which it is the type species. *Thyrostroma compactum* (Sacc.) v. Höhnelt now seems to be the logically tenable binomial. It is not clear what connection, if any, exists between this and the name *Coryneum compactum* B. and Br. that appears in literature.

Since May 17, 1935, when the *Thyrostroma* canker was removed, the tree has been observed frequently, and, to date, there has been no further appearance of infection, indicating that excision may be a satisfactory method of control. Numerous *Ulmus pumila* L. trees were examined throughout Illinois during 1935, but were not found affected with this

¹ Saccardo, P. A. *Fungi Veneti novi vel critici*. Ser. V. *Nuovo Gior. Bot. Ital.* 8: 198. 1876.

² Saccardo, P. A. *Fungi Veneti novi v. critici v. mycologiae Venetae*. Addendi . . . Ser. XIII. *Michelia* 2: 542. 1882.

³ Höhnelt, F. von. *Fragmente zur Mykologie*. Sitzber. k. Akad. Wiss. Wien Math. Naturw. Kl. 116: 154. 1907.

⁴ Höhnelt, F. von. *Fragmente zur Mykologie*. Sitzber. k. Akad. Wiss. Wien Math. Naturw. Kl. 120: 472. 1911.

canker.—J. C. CARTER, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana, Illinois.

A Die-back of Douglas Fir.—During the summer of 1935, specimens of a die-back of Douglas fir (*Pseudotsuga taxifolia* Brit.) were received for examination at the New Haven Office of the Division of Forest Pathology, U. S. Department of Agriculture. The leaves of the current season's growth were pale yellow with small black pycnidia at the base or scattered the entire length of the needles. Most of these pycnidia contained brown unicellular spores, but a few hyaline unicellular and brown bicellular spores were among them. Since the spores were predominantly unicellular, the fungus was identified as a species of *Sphaeropsis*.

Inspection of affected trees, located on an estate at Southold, Long Island, showed conspicuous infection of terminal growth on the lower branches. The young twigs had been killed back to the nodes, but no defoliation had occurred. The leaves of the previous season's growth showed no indication of disease. On the twigs, however, definitely defined cankers with small erumpent pycnidia of the fungus extended back from the nodes to about one-half the length of that year's growth. None of the Douglas firs in the planting were entirely disease-free, although some were much more heavily infected than others.

Several Austrian pines, *Pinus nigra* Arn.; a white pine, *P. strobus* L.; and a Colorado blue spruce, *Picea pungens* Engelm., near the Douglas firs, showed a similar die-back and infection by a species of *Sphaeropsis*. A study of material from the 4 hosts indicated that the pycnidia, spores, and cultural characteristics of the organisms isolated were very similar. Measurements of 50 brown unicellular spores from pycnidia on the needles of each of these hosts showed a range of $24-40\ \mu \times 9-15\ \mu$. The length of the majority of the spores fell within the following ranges: Austrian pine $30-37\ \mu$, Douglas fir $26-31\ \mu$, white pine $28-34\ \mu$, spruce $26-30\ \mu$. Spores from pycnidia on the twigs of Douglas fir corresponded closely with those from Austrian pine, 50 brown unicellular spores of the former showing a range of $27.6-38.8\ \mu$, the majority being within the range $29-35.6\ \mu$. This difference may be due to variation in spore maturity.

Numerous reports have been made of the occurrence of a twig and needle blight attacking various species of 2-, 3-, and 5-needle pines, including *Pinus nigra*, *P. strobus*, and *P. sylvestris* L., with which is associated a fungus identified as *Sphaeropsis ellisii* Sacc.^{1,2} or as *Diplodia pinea* (Desm.) Kickx

¹ Hedgcock, G. G. Notes on the distribution of some fungi associated with diseases of conifers. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. 16: 28-42. [Mimeographed.] 1932.

² White, R. P. Notes on new or unusual outbreaks of diseases of ornamentals in New Jersey in 1935. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. 20: 79-81. 1936.

(*Botryodiplodia pinea* (Desm.) Petr.).^{3,4} No disease of Douglas fir caused by a species of either of these genera has been reported in the United States, so far as known. The descriptions of the disease and the accompanying fungus on the pines, however, seem closely applicable to the disease and fungus of the Douglas firs. A specimen of this latter host from near St. Helena, California, showing a definite canker, with pycnidia⁵ at the node of a living branch, was collected by J. S. Boyce in July, 1930. The pycnidia and spores on this canker correspond with those on the Long Island specimens, measurements of the brown unicellular spores showing a range of $26-35.6 \times 9.6-14.8 \mu$.

A similar disease of Douglas fir has been reported from Italy by Petri⁶ who suggested that it had spread to the firs from neighboring Scotch pines. In New Zealand⁷ the death of Douglas fir saplings in a young exotic forest has been attributed to infection by *Diplodia pinea* following a protracted drought.

Petri stated that the susceptibility of the Douglas firs in Italy was correlated with unfavorable growing conditions, such as excessive moisture, insufficient light, and low temperatures. Although these particular conditions would not be applicable to the trees in the Long Island location, it is undoubtedly true that the situation, in a light sandy soil and outside the natural range for Douglas fir, is inimical to normal development, and that the trees may have been weakened by drought, followed by unusually severe winter conditions.—ALMA M. WATERMAN and J. ARMSTRONG MILLER, Division of Forest Pathology, Bureau of Plant Industry. In cooperation with the Osborn Botanical Laboratory, Yale University, New Haven, Conn.

Cytosporina ludibunda on American Elm.—During the summer of 1934 and, to a lesser degree, that of 1935, *Cytosporina ludibunda* Sacc. was observed to be prevalent on American elms in Illinois. Attacked trees exhibited a wilting and dying back of the diseased branchlets, followed by the formation of cankers when infection reached larger and older parts. Fruiting of the fungus occurred in the cankered areas. Successful inoculations have been made with pure cultures of the fungus on potted American elms.

³ Schwarze, C. A. The parasitic fungi of New Jersey. New Jersey Agr. Expt. Sta. Bull. 313. 1917.

⁴ Curtis, K. M. A die-back of *Pinus radiata* and *P. muricata* caused by the fungus *Botryodiplodia pinea* (Desm.) Petr. New Zealand Inst. Trans. and Proc. 55: 52-57. 1926.

⁵ Identified as a species of *Sphaeropsis* by G. G. Hahn, Division of Forest Pathology, Bureau of Plant Industry, U. S. D. A.

⁶ Petri, L. Disseccamento dei rametti di *Pseudotsuga Douglasii* Carr. prodotto da una varietà di *Sphaeropsis ellisii* Sacc. Ann. Mycol. 11: 278-880. 1913.

⁷ New Zealand. State Forest Serv. Ann. Rept. Dir. Forestry for the year ended March 31, 1934. 17 pp. 1934. Abs. in Rev. Appl. Mycol. 14: 65. 1935.

A series of 5 trees were inoculated between March 6 and May 14, 1935. All inoculations were made on terminal foliage and wood as the buds were opening. The leaves and wood were sterilized with 1/1000 HgCl₂ and rinsed in sterile water. On 3 trees, the sterilized leaves and twigs were injured by pricking with a sterile needle previous to inoculation, to provide a direct avenue of entrance for the fungus, while on 2 trees the sterilized parts were not injured. In each case, a test tube containing a growing culture of *Cytosporina ludibunda* Sacc. on slanted potato-dextrose agar was placed over the twig to be inoculated and held with a ring stand, so that the fungus colony was kept in contact with the sterilized leaves and wood. The test tube was plugged with cotton to prevent contamination.

After intervals ranging from 25 to 54 days, signs of successful inoculation became apparent and symptoms of disease continued to develop through varying periods, the first tree wilting completely in 54 days and the last after the lapse of 7 months. Reisolations were attempted, first, when death of inoculated twigs had progressed downward 8 to 10 inches from the region exposed to inoculation and, second, when the trees had wilted completely. From each tree platings on corn-meal agar were made of tissue taken at points 4, 8, and 12 inches below the place of inoculation and 4 inches above the soil line.

The uninjured trees, though having shown external signs of successful inoculation, presented no internal symptoms of infection, and the attempts to reisolate the *Cytosporina* from them were all unsuccessful.

The injured trees, however, gave approximately the same symptoms observed in trees naturally infected, including particularly the light brown discoloration of parts of the xylem. Attempts to reisolate the fungus from these trees were successful. From one the *Cytosporina* was secured 8 inches below the point of inoculation; from another, at the base and at 12 inches, but not at 4 or 8 inches, below the point of inoculation; and from the third at all 4 of the points tried.—J. C. CARTER, Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana, Illinois.

BOOK REVIEW

Wollenweber, H. W., and O. A. Reinking. *Die Verbreitung der Fusarien in der Natur*. 80 pp., illus. R. Friedländer und Sohn. Berlin. 1935.

The book consists of an introduction, 8 lists of Fusaria in cross-index form and in different groups; and a chapter, with 12 figs., on genera of fungi other than Fusarium, some members of which formerly had been included in Fusarium, but no longer can be retained there. In the introduction are briefly described the biology, the nature of former and present records of occurrence, systematic relationship with other Hypho- and Ascomycetes, the relative parasitism and saprophytism, the relative usefulness of former and present host records, and of occurrence in the air and soil, of the fungi of the genus Fusarium. The systematic details are omitted in this book because these just recently have been published in another text¹ by the same authors.

The list of Fusaria given in this book are as follows: (1) Of all names of Fusarium species and varieties, with forms, now accepted by the authors, and with corresponding Ascomycetes and references to the illustrations given in Wollenweber's *Fusaria autographice delineata*, tables 1-1200; (2) of Fusaria arranged in sections and subsections, with citations to the 28 illustrations given in this book; (3) of Fusaria occurring on plants, fungi included, on living animals, on raw materials and products of plants and animals, and also found in soil, air, and water—here under each of the Fusaria, are given the hosts, substrata, and media in or on which the fungus has been found. The 5 remaining lists are those of special groups of Fusaria—(4) on higher plants, given under generic names (about 400) of the hosts alphabetically arranged; (5) on algae, lichens, and mosses; (6) on other fungi; (7) on animals; and (8) on raw materials and products of plants and animals, also in soil, air, and water.

The subject is presented in this book apparently with remarkable thoroughness and is brought up to date. The references given here, in the list of Fusaria, to the 1200 illustrations in Wollenweber's *Fusaria autographice delineata*, and the fact that here the list of the finally accepted names is identical with that in *Die Fusarien*, make this little book very valuable to all interested in these fungi. In a way, it completes the previous works of Wollenweber and makes his *Fusaria autographice delineata*, and also his and Reinking's *Die Fusarien*, directly and more readily usable. It should be stated also that the general lists of the fungi and hosts, and of the groups of them, provide extremely convenient means to locate quickly previous records of the fungi.—C. D. SHERBAKOFF, University of Tennessee, Knoxville, Tenn.

¹ Wollenweber, H. W., und O. A. Reinking, *Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung*. 355 pp., Paul Parey, Berlin, 1935.



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HEAT TREATMENTS FOR THE CURE OF YELLOWS AND OTHER VIRUS DISEASES OF PEACH¹

L. O. KUNKEL

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INTRODUCTION

Curing peach trees of the yellows disease by incubating them at approximately 35° C. for two weeks or longer was reported in 1935 (10). Further studies on the effectiveness of moderately high temperatures for the cure of yellows have now been completed. Since the virus of this disease is not transmitted mechanically, except by grafting, its resistance to high temperatures can not be studied by means of techniques usually employed in work on viruses transmitted by ordinary mechanical inoculations. The fact that yellows virus can be inactivated by exposure of trees to temperatures that cause little or no injury to peach tissues makes an *in vivo* study of its thermal relationships possible. Since most plant viruses are not destroyed by heat treatments sublethal for their hosts, it seemed worthwhile to determine with some accuracy the sensitiveness of yellows virus to heat inactivation at several different temperatures. It also seemed desirable to study the effects of heat treatments on other virus diseases of peach at this time, since experiments including tests of other diseases frequently could be carried out almost as easily as experiments with yellows alone. The results obtained are reported in this paper.

MATERIALS AND METHODS

The trees used in heat-treatment studies were grown under conditions similar to those described in an earlier paper (11) from seed purchased of a dealer at Germantown, Pennsylvania. All seedlings were selected for uniformity in size, vigor, and type of growth, and were less than 2 years old when taken for experiment. The yellows virus used was the same as that mentioned in the paper to which reference has already been made.

Three different methods were employed in studying the effects of high temperature on diseased peach tissues. The first method involved the incu-

¹ Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

bation of actively growing diseased trees in a thermostatically controlled room lighted by means of a window. The trees were placed on a table directly in front of the window. The room was held at a temperature varying from about 34.4° C. to 36.3° C. After incubation in the room for varying periods of time, the trees were removed to a greenhouse and kept under observation. When they had produced normal-appearing foliage and a healthy type of growth over a period of a year or more, they were considered to have been cured. Subinoculations from such trees invariably failed to cause disease. This method of treatment was satisfactory in many respects, and was used in the cure of about 300 trees. The chief disadvantage of the method was that it required long periods of incubation. Attempts to shorten the periods by raising the temperature proved impracticable, because of the burning of foliage and tender young branches. Another disadvantage of the method was that the virus was not inactivated at a uniform rate in different parts of the trees. It was destroyed in from 4 to 6 days in slender young branches, in about 2 weeks in thick stems and branches, and in from 2 to 4 weeks in large roots. Trees with virus persisting only in their roots had the appearance of being cured and produced healthy-looking foliage for several months following treatment, but symptoms of disease would then appear in shoots produced near the soil level and the virus would spread upward into the tops. This lack of uniformity in rate of destruction of virus in roots and tops is believed to have been due chiefly to cooling caused by evaporation of water from the moist soil in which the roots were imbedded.

In order to avoid the difficulty encountered in curing roots, infected trees were sometimes treated before virus had had time to spread from the point of inoculation into the roots. In these instances the trees were always treated while the disease was in an early stage of incubation, *i.e.*, within from 3 to 10 days after inoculation. This modification of the method gave fairly uniform results and eliminated the difficulty of having virus persist in roots after a treatment that had cured the tops, but it had the disadvantage of requiring application of treatment before the appearance of symptoms.

The second method consisted in the immersion of dormant trees for a short period of time in a tank of water held at about 50° C. The trees were removed from the pots in which they had been grown and the earth washed from their roots. After treatment they were repotted in fresh soil. This method was highly effective for the cure of yellows, but could be used only on trees in a dormant condition. While it was satisfactory for curing trees, it was not well adapted for studying virus inactivation. Even with this method of treatment yellows virus was more readily destroyed in slender than in thick stems and branches. The lack of uniformity in rate of destruction of virus in stems of different diameters was of little consequence when the object in view was to effect a cure, but it became important when tests

were undertaken to determine the effectiveness of treatments at any given temperature. Since it seemed desirable to eliminate, as far as possible, this source of error in studying the thermal relationships of the yellows virus, a third method of treating diseased tissues was adopted. It consisted in immersing pieces of yellows branches about 5 inches long and $\frac{3}{32}$ of an inch in diameter in a thermostatically controlled water bath in which the water was kept in constant motion by means of an electrically propelled stirrer. All leaves were removed from the branches before treatment. After exposing the cuttings for varying periods of time, one or more buds were removed from each and inserted in a healthy young tree. If virus in the buds had been destroyed, the tree remained healthy; if not, it came down with yellows. When bud sticks of current season's growth were carefully selected for uniformity in thickness, hardness, and maturity, this method gave fairly consistent and reproducible results. It was employed in most of the experiments to be reported.

Rosette and little-peach viruses used in heat-treatment experiments were from the sources mentioned in another paper (11). Red-suture virus was obtained from Donald Cation, of East Lansing, Michigan. All of the 3 methods described above were used in treating the diseases caused by these viruses.

EXPERIMENTAL

Appearance of Cured Trees

When a tree having yellows was incubated in the hot room described above for a period of time sufficiently long to destroy all the virus in its tissues and was then placed in a greenhouse and held under favorable conditions, the growing buds produced only healthy-appearing leaves and stems. As is well known, the branches of yellows trees assume a more upright habit of growth than is normal for healthy trees. When the upright-growing lateral branches were cured, they at once began to produce less upright-growing tips and all side branches formed subsequent to the cure grew out in the spreading manner characteristic of healthy trees. Figure 1 shows a side branch from a tree cured of yellows by heat treatment. The lower portion of the branch was produced while the tree was diseased, the upper portion after it was cured. The location of the terminal bud at the time the tree was subjected to heat treatment is shown by the angle that marks the change in direction of growth of the branch. On being cured, the branch immediately changed its habit of growth, as well as the type of leaves and stem internodes it produced. Subinoculations from both the upper and lower portions of the branch failed to transmit disease. The effect of yellows on direction of growth is shown in a more striking manner by figure 2, which presents a portion of the main stem of a young tree with a side branch

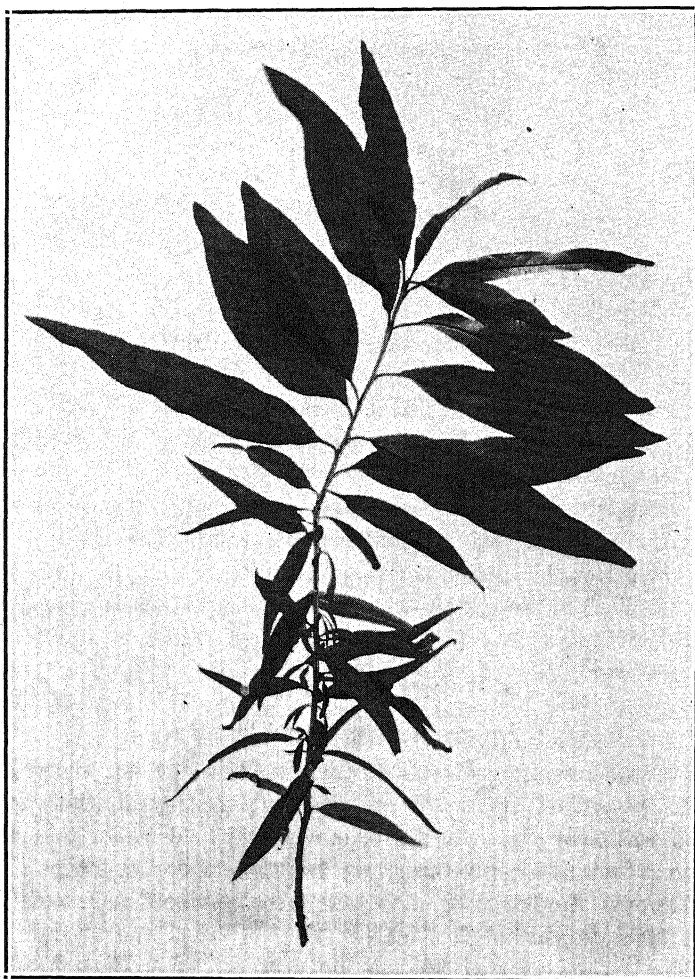


FIG. 1. Lateral branch from peach tree cured of yellows. The lower portion of the branch was produced before and the upper portion after the tree was cured. The lower portion bears small narrow chlorotic leaves that are typical for yellows disease, while the upper portion bears large deep green leaves separated by internodes of normal length. The angle made by the two sections marks the location of the terminal bud at the time of cure.

attached. The tip of both stem and branch was removed in order to reduce the size of the specimen to be photographed. The lower part of the branch bears no leaves. This section was produced while the tree was healthy. The angle between this first section and the stem is normal for a healthy tree. The second or middle section of the branch bears small narrow leaves and shows the upright habit of growth characteristic of side branches affected by yellows. This section was produced after the tree became diseased. The

third section of the branch bears large green leaves. A straight line drawn through the long axis of this portion to the main stem would subtend an angle approximately equal to that subtended by the first section. The third section was produced after the tree was cured by heat treatment. The terminal bud of this branch produced normally-directed growth both before it became diseased and after it was cured. It produced abnormally-directed growth while diseased. Subinoculations made from each of the 3 sections shortly after the photograph was taken caused no infections and proved that the branch was virus-free throughout. The tissues formed while the branches shown in figures 1 and 2 were diseased did not lose the symptoms of yellows after the branches were cured. The small, narrow leaves and

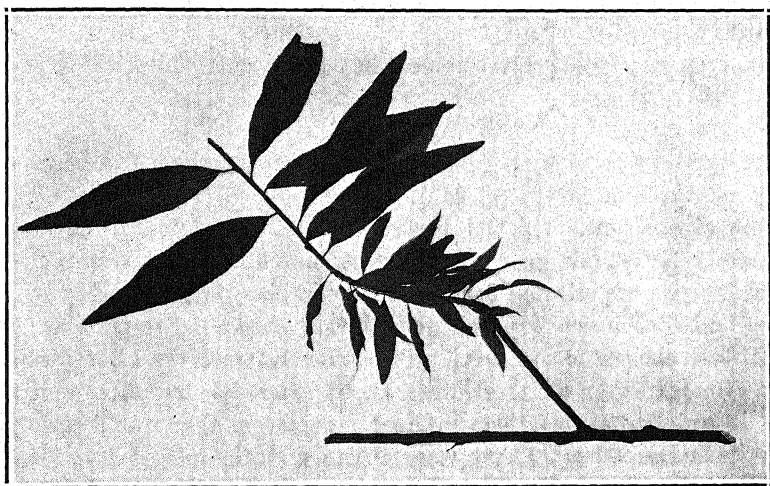


FIG. 2. A portion of the main stem of a young peach tree with side branch attached. The lower section of the side branch was produced while the tree was healthy, the middle section while it was diseased, and the upper section after it was cured. The terminal bud of the branch produced normally-directed growth both before the tree became diseased and after it was cured. The bud produced abnormally-directed growth as well as small leaves and short internodes while the tree was diseased.

short internodes produced while virus was present retained the light yellow color that they had before treatment. The new growth from cured trees bore no symptoms of yellows. Only virus of the masked-symptom type could have been present. If the treatments merely attenuated yellows virus, one would expect that different exposures would attenuate it to different degrees, and that some attenuated strains would produce mild but visible symptoms of disease. No evidence of this was obtained. It is known that weak strains of tobacco mosaic protect against ordinary or severe strains of this disease (9), and likewise that little-peach disease protects against

yellows (11). If trees that have been cured of yellows by heat treatments, or trees that have been inoculated with buds from cured trees were affected by an attenuated yellows virus, they would be expected to show immunity from the type of yellows caused by unattenuated virus. Accordingly, cured trees and trees in which buds from cured trees had been inserted were inoculated with yellows buds. In all cases the trees came down promptly and showed typical symptoms of yellows. If any unattenuated virus had been present in trees cured of yellows, it should have multiplied, for, as has been proved experimentally, it does multiply in such trees when they are reinfected. Moreover, if unattenuated virus had been present, it should have been transmitted when subinoculations were made to healthy trees. As no evidence was obtained that virus either multiplied in, or could be transmitted from, cured trees, it is concluded that none was present, and that cure resulted from complete destruction of the causative agent.

Heat Treatments for the Cure of Yellows

Since yellows trees were first cured by exposing them for long periods of time at approximately 35° C., a description will be given of a typical hot-room experiment. On October 1, 1934, 60 healthy seedling trees approximately 3½ feet tall, grown from seeds planted in pots during the preceding February, were selected for uniformity in size and vigor of growth. A yellows bud was inserted in the stem of each of 50 of the trees at a point about 2 feet above the soil level. The other 10 trees were not inoculated. About 1 month later all trees were taken from the greenhouse to a cold frame, in order to expose them to low temperatures that would induce dormancy. On December 20 they were returned to the greenhouse. By February 1, 1935, all of the trees had produced new growth, and all except 1 of those that were inoculated showed characteristic symptoms of yellows. On examination it was found that the bud used to inoculate the tree that failed to become infected had died. The buds used in inoculating the other 49 trees had lived. On February 7, 1935, 27 of the yellows trees were placed in the hot room described above. The other 22 diseased trees and the 10 healthy trees were left in the greenhouse to serve as controls. On each succeeding day, for a period of 27 days, one of the treated trees was returned to the greenhouse. All of the trees in this experiment were again transferred to a cold frame on September 11, 1935. On December 16 they were brought back into the greenhouse and forced into growth. The trees were held under observation for 14 months following treatment. Their appearance with respect to yellows symptoms after different intervals of time is shown in table 1. All trees kept in the hot room for 10 days or longer produced healthy-appearing new growth after the treatment. Those exposed for less than 10 days produced new growth bearing symptoms of yellows.

TABLE 1.—*Cure of peach yellows by incubation of trees in a hot room held at 34.4 to 36.3° C.*

Period of exposure	Appearance of trees after					
	54 days	81 days	105 days	215 days	365 days	420 days
1 day	D	D	D	D	D	D
2 days	D	D	D	D	D	D
3 "	D	D	D	D	D	D
4 "	D	D	D	D	D	D
5 "	D	D	D	D	D	D
6 "	D	D	D	D	D	D
7 "	D	D	D	D	D	D
8 "	D	D	D	D	D	D
9 "	D	D	D	D	D	D
10 "	H	D	D	D	D	D
11 "	H	D	D	D	D	D
12 "	H	D—	D—	D	D	D
13 "	H	D—	D—	D	D	D
14 "	H	H	H	H	D	D
15 "	H	D—	D—	D	D	D
16 "	H	D—	D—	D	D	D
17 "	H	H	H	D—	D	D
18 "	H	H	H	D—	D	D
19 "	H	H	H	H	H	H
20 "	H	H	H	H	D	D
21 "	H	H	H	H	D	D
22 "	H	H	D—	D—	D	D
23 "	H	H	D—	D—	D	D
24 "	H	H	H	H	H	H
25 "	H	H	H	H	H	H
26 "	H	H	H	H	H	H
27 "	H	H	H	H	H	H

H = Healthy;

D = Diseased;

D— = Disease symptoms only in lower part of tree

After varying periods of time, as is indicated in table 1, yellows symptoms appeared in twigs produced on the stems of many previously healthy-looking treated trees. Such twigs always appeared first at or near the soil level. Subinoculations made from different parts of these trees proved that their healthy-appearing tops were free of virus but that their lower portions were infected. All healthy check trees remained healthy, and all diseased check trees diseased. Only 5 of the 27 treated trees were permanently cured. These 5 trees were exposed for periods of 19, 24, 25, 26, and 27 days, respectively. No explanation can be given as to why the tree exposed for 19 days was cured, while trees exposed for 20, 21, 22, and 23 days, respectively, were

not cured. Similar irregularities were observed in other heat-treatment experiments. The question might be raised as to whether or not the 5 trees that were still healthy in appearance 14 months after they were treated might not eventually come down with yellows. In the writer's experience, all treated trees that have remained free of symptoms after a vegetative period as long as 8 months are permanently cured. Eleven trees that were heat-treated in an earlier experiment showed no signs of disease 26 months after they were removed from the hot room. Subinoculations made from both stems and roots of 2 of the trees approximately 1 year after treatment failed to transmit yellows. The experiments prove that yellows trees can be cured by prolonged exposure to air held at about 35° C.

Trees as old as those used in the experiment described above were not seriously injured by hot-room exposures lasting as long as 40 days, but younger trees were unable to survive such prolonged treatments. As was stated in the description of methods, trees are more readily cured when treated before virus has had time to spread into their roots than when treated after roots are infected. Tender young trees can be cured of yellows very readily if they are treated shortly after infection.

In order to present data illustrating these facts, another heat-treatment experiment that was started at the same time as that just reported will be described. Forty-eight healthy seedling trees about 6 months old and approximately 18 inches high were selected for uniformity in size and vigor. Each of 45 of the trees was inoculated by inserting a yellows bud in the stem at a point 6 to 8 inches above the soil level. The other 3 trees were not inoculated and served as controls. Three days later 42 of the 45 inoculated trees were placed in the hot room. The other 3 inoculated trees were left in the greenhouse to serve as controls. At daily intervals over a period of 2 weeks, 3 trees were transferred from the hot room to the greenhouse. Shortly after the treatments were completed, all trees except those killed by heat were placed in a cold frame to promote dormancy. They were taken back to the greenhouse during the last week in December and were held under observation until October 1, 1935, when the experiment was ended. All of the trees exposed to treatment for 6 days or longer, and 1 of the trees exposed for only 5 days, died. The buds used in inoculating lived in all trees that survived treatment. The trees that were treated for 1, 2, and 3 days came down with yellows, as did the 3 inoculated untreated control trees. The 3 that were treated for 4 days and the 2 that survived 5 days of treatment remained healthy. The 3 noninoculated control trees likewise remained healthy. A tree from each of the sets that survived treatment is pictured in figure 3. The photograph was taken February 16, 1935, and shows the trees as they appeared 4½ months after treatment. The 3 diseased trees standing on the left in the picture were exposed for 1, 2, and 3

days, respectively, while the 2 healthy trees standing on the right were exposed for 4 and 5 days, respectively. Under the conditions of this experiment, the yellows virus was destroyed by treatments lasting 4 and 5 days, but not by treatments lasting 3 days or less. The experiment proves that yellows virus in newly-infected young trees is destroyed by hot-room treatments lasting about 4 days.

It was thought that successive treatments involving 1- or 2-day exposures in the hot room might prove effective for the cure of yellows and cause less injury to trees than prolonged continuous exposures. The following experiment was made for the purpose of testing this possibility. Twelve trees that had been affected by yellows for about 2 months were divided into 4 sets of 3 trees each. On April 30, 1935, 3 of the sets were placed in the hot room. The 4th was not treated and served as a control. The 1st set was returned to the greenhouse after continuous incubation in the hot room for 30 days. The 2nd set was treated by incubating the trees in the hot room on alternate days during a period of 60 days. The 3rd set was similarly treated during alternate 2-day periods. The trees were held in a greenhouse on the days they were not in the hot room. The total length of time each set of treated trees was kept in the hot room amounted to 30 days, but the 2nd set was exposed during 30 different 1-day periods, while the 3rd set was exposed during 15 different 2-day periods. All trees were held under observation for a year following treatments. Those that were kept in the hot room continuously for 30 days were cured, but none that were subjected to intermittent treatments were cured or even favorably affected insofar as could be judged by symptoms. However, they suffered no injury from the treatments. The effectiveness of successive treatments lasting longer than 2 days was not investigated.

An experiment designed to determine the period of treatment necessary for the curing of dormant trees by the second method will now be described. The experiment was planned after preliminary tests had shown the approximate time required to inactivate virus at 50° C. Eight of a group of 10 healthy young trees about 11 months old were inoculated with yellows virus by inserting a yellows bud in the stem of each tree at a point about 2 feet above the soil level on September 3, 1934. All of the inoculated trees showed symptoms of yellows within 6 weeks. The 2 noninoculated control trees remained healthy. During the first week in November the trees were transferred from the greenhouse, in which they had been grown, to a cold frame, where they were allowed to become dormant. On February 12, 1935, they were returned to the greenhouse. Soil was removed from their roots 2 days later, after it had had time to thaw. Six of the trees were then treated by immersing them for periods of time varying by 2-minute intervals from 2 to 12 minutes in a tank of water held at approximately 50° C. The other 2

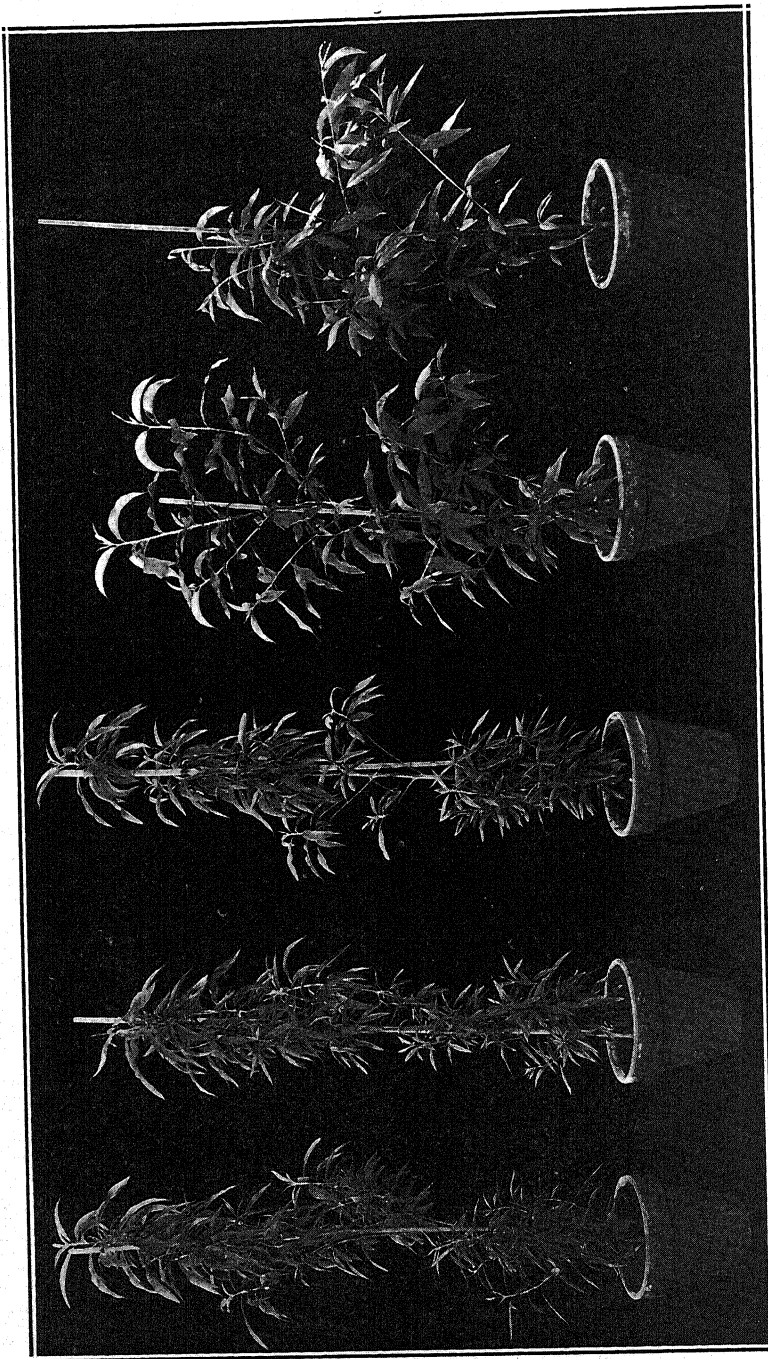


FIG. 3. Five trees used in a hot-room experiment. All of the trees were infected with yellows virus before treatment. The 3 trees shown on the left were treated for 1, 2, and 3 days, respectively; the 2 trees on the right for 4 and 5 days, respectively. The picture shows that the latter were cured and that the former were not cured.

TABLE 2.—*Thermal inactivation points of peach-yellow virus in bud sticks*

34.35° C.			38° C.		40° C.		42° C.			44° C.		46° C.		48° C.		50° C.						52° C.		54° C.		56° C.	
Time	Effect		Time	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes		
	Effect	Effect																									
1 hr.	+	+	3 hrs.	+	20	+	25	+	18	+	5	+	5	+	2	+	1	+	1	+	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	
5 hrs.	+	+	4 "	+	30	+	30	+	21	+	10	+	10	+	4	+	2	+	2	+	1	0	$1\frac{1}{2}$	0	$1\frac{1}{2}$	0	
10 "	+	+	5 "	+	40	+	35	+	24	+	15	+	15	+	6	+	3	0	3	0	$1\frac{1}{2}$	0	$1\frac{1}{2}$	0	$1\frac{1}{2}$	0	
20 "	+	+	6 "	+	50	+	40	0	27	+	20	+	20	+	8	0	4	0	4	0	2	0	2	0	1	0	
30 "	+	+	7 "	+	60	+	45	0	30	+	25	+	25	+	10	0	5	0	5	0	$2\frac{1}{2}$	0	$2\frac{1}{2}$	0	$1\frac{1}{2}$	0	
2 days	+	+	8 "	+	70	+	50	0	33	+		+	12	+	12	0	6	0	6	0	3	0	3	0	$1\frac{1}{2}$	0	
3 "	+	+	9 "	+	80	+	55	0	36	+		+	14	0	14	0	8	0	7	0	$3\frac{1}{2}$	0	$3\frac{1}{2}$	0	$1\frac{1}{2}$	0	
4 "	0	0	10 "	+	90	+	60	0	39	0					16	0	10	0	8	0	0	0	0	0	$1\frac{1}{2}$	0	
5 "	0	0	11 "	0	100	+	65	0	42	0					18	0	12	0	9	0					$1\frac{1}{2}$	0	
6 "	0	0	12 "	0	110	+	70	0	45	+					20	0	14	0	10	0					$1\frac{1}{2}$	0	
7 "	0	0	13 "	0	120	+	75	0	48	0					22	0	16	0	0	0					2	0	

+ indicates that bud transmitted disease;

0 indicates that bud did not transmit disease.

yellowed trees and the 2 healthy control trees were not treated. Immediately after treatment, all trees were repotted and placed in a greenhouse. A month later the tree held in the bath for only 2 minutes showed yellowed symptoms to the same extent as the untreated yellowed trees; but the other 5 treated trees appeared to be healthy. Two and one-half months after treatment, the 3 trees held in the bath for 4, 6, and 8 minutes, respectively, showed symptoms of yellowed. The tree exposed for 4 minutes was thoroughly diseased, but the tree exposed for 6 minutes showed yellowed symptoms only in shoots from the stem and large branches, and the tree exposed for 8 minutes only in shoots from the main stem. The 2 trees exposed for 10 and 12 minutes, respectively, were held under observation for a period of 13 months following the treatment. These trees grew vigorously and never showed symptoms of yellowed. The experiment proves that, under the conditions described, dormant trees may be cured by treatments lasting 10 minutes or longer.

The third method of treatment described above was employed in experiments designed to determine the time required for thermal inactivation of virus at a number of different temperatures. Table 2 presents a summary of the results obtained in some of these experiments. The temperatures employed varied from 34.35° C. to 56° C. The low temperature was approximately the same as the minimum temperature of the hot room in which trees treated by the first method were exposed. At such a low temperature an exposure of from 4 to 5 days was necessary to inactivate virus in the buds of treated sticks. It was inactivated by a 4-day exposure in the first, but not in the second experiment. The reason for this variability is not definitely known, but as temperature was accurately controlled, it is presumed to have been due to differences inherent in the bud sticks. Similar irregularities appear in the data from some of the other experiments. At 38° C. virus was inactivated in 11 hours, but not in 10 hours or in shorter periods of time. The exposure periods employed with bud sticks held at 40° C. and at 44° C. were not sufficiently long to inactivate virus. These tests show that 2 hours at 40° C. and 25 minutes at 44° C. were insufficient for inactivation. Some other experiments carried out at the same temperatures, but under conditions that gave less accurate control than was had in the experiments reported in table 2, indicate that only slightly longer treatments would have given inactivation at 40° C. and 44° C.

The effectiveness of other temperatures for the destruction of virus is shown by the results of the other experiments summarized in the table. A 40-minute treatment was sufficient at 42° C., a 15-minute treatment at 46° C., a 14-minute treatment at 48° C., a 1-minute treatment at 52° C., and a 1½-minute treatment at 54° C. The table shows that at 54° C. virus was destroyed by a ½-minute treatment but not by a 1-minute treatment. A

somewhat similar inconsistency appears in the data from one of the experiments in tests at 42° C. At 56° C. virus was destroyed by treatment for $\frac{1}{4}$ minute, which was the shortest period tested. The results of 4 experiments on inactivation time at 50° C. are reported in the table. Virus was destroyed by a 4-minute exposure but not by a 2-minute exposure in each of these experiments. It was destroyed by a 3-minute treatment in 2 experiments but not in a third.

The shortest inactivation periods obtained at several different temperatures are indicated by points used in plotting the curve presented in figure 4, in which a logarithmic scale was employed to show time in hours on the abscissa and an arithmetical scale to show temperature in degrees centigrade on the ordinate. The curve is steeper at high than at low temperatures. This indicates that inactivation of yellows virus in living bud tissues is not governed by a linear function. The minimum temperature at which yellows buds can be cured has not yet been determined.

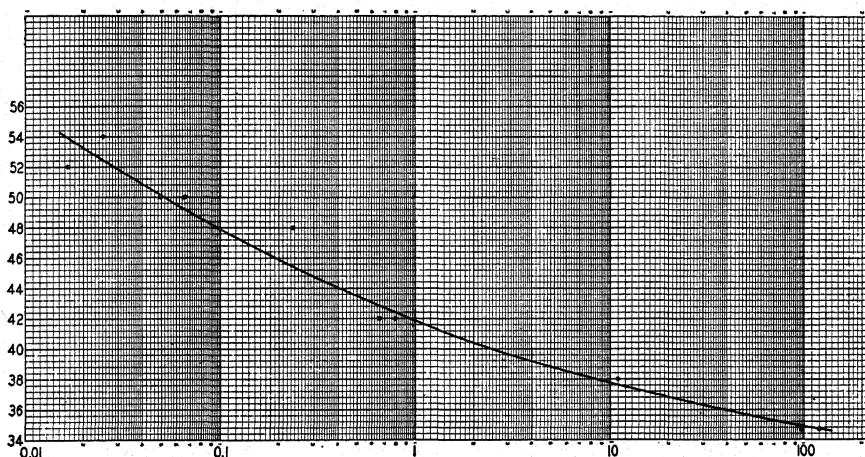


FIG. 4. Curve showing the relation of temperature and time of treatment to thermal inactivation of yellows virus in bud sticks. A logarithmic scale is employed to show time in hours on the abscissa and an arithmetical scale to show temperature in degrees centigrade on the ordinate.

The thermal inactivation time for yellows virus at the different temperatures investigated was found to be much shorter than the time required to kill bud tissues. In fact, the exposure periods that bud tissues were able to endure at high temperatures were so much greater than the thermal inactivation time of virus that no difficulty was experienced in curing buds without injuring them. The interval between the minimum inactivation period of the virus and the maximum endurance period of buds was greater at low than at high temperatures. Two experiments carried out at approxi-

TABLE 3.—*Inactivation time of yellow virus and endurance time of diseased buds at 45° and 50° C.*

Treatment at approximately 45° C.				Treatment at approximately 50° C.		
Exposure time	Effect on virus	Effect on bud	Exposure time	Effect on virus	Effect on bud	Exposure time
5 mins.			170 mins.			2 mins.
10 "	+	bud alive	180 "	0	bud alive	4 "
15 "	+	" "	190 "	0	" "	6 "
20 "	+	" "	200 "	0	dead	8 "
25 "	+	" "	210 "	0	alive	10 "
30 "	0	" "	220 "	0	" "	12 "
35 "	0	" "	230 "	0	" "	14 "
40 "	0	" "	240 "	0	" "	16 "
45 "	0	" "	250 "	0	" "	18 "
50 "	0	" "	260 "	0	" "	20 "
55 "	0	" "	270 "	0	" "	22 "
60 "	0	" "	280 "	0	" "	24 "
65 "	0	" "	290 "	0	" "	26 "
70 "	0	" "	300 "	0	dead	28 "
75 "	0	" "	310 "	0	alive	30 "
80 "	0	" "	320 "	0	" "	32 "
85 "	0	" "	330 "	0	" "	34 "
90 "	0	" "	340 "	0	dead	36 "
95 "	0	" "	350 "	0	alive	38 "
100 "	0	" "	360 "	0	dead	40 "
105 "	0	" "	370 "	0	alive	42 "
110 "	0	" "	380 "	0	" "	44 "
115 "	0	" "	390 "	0	dead	46 "
120 "	0	" "	400 "	0	alive	48 "
125 "	0	" "	410 "	0	" "	50 "
130 "	0	" "	420 "	0	" "	52 "
135 "	0	" "	430 "	0	" "	54 "
140 "	0	" "	440 "	0	dead	56 "
145 "	0	" "	450 "	0	" "	58 "
150 "	0	" "	460 "	0	" "	60 "
155 "	0	" "	470 "	0	" "	62 "
160 "	0	" "	480 "	0	" "	64 "
165 "	0	" "	490 "	0	" "	66 "

+ indicates that bud transmitted disease.

0 indicates that bud did not transmit disease.

mately 45° C. and 50° C., respectively, will be described in order to show the effect of temperature on the length of this interval. The accurately controlled water bath used in the tests reported in table 2 was not available at the time these experiments were made. The heat treatments were carried out in a jar of water held in an electric oven. The temperature at which the water was maintained varied somewhat during both experiments. However, it did not go more than $\frac{1}{2}$ ° C. above or below the desired temperature at any time. The results obtained are shown in table 3. Yellows virus was destroyed by a 25-minute treatment at 45° C. and by a 4-minute treatment at 50° C. All buds treated for 3 hours or less at 45° C. survived and grew on the trees into which they were transplanted. At this temperature buds were not invariably killed by exposure periods of less than about 7 hours. At 50° C. all buds treated for periods of 24 minutes or less survived, and all treated for 30 minutes or longer died. Somewhat erratic results with respect to the length of time that buds were able to survive treatment are shown by both experiments. The buds exposed at 45° C. for periods of 190, 300, 340, 360, and 390 minutes, respectively, died, while all of the other buds held at the same temperature for a period of 430 minutes or less survived. Similarly, the bud held at 50° C. for 28 minutes survived, while that held at the same temperature for 26 minutes died. No explanation can be offered for this variability in survival time of buds. The experiments show clearly that diseased peach tissues are able to endure much longer treatments than are necessary for the destruction of yellows virus. No evidence was obtained that tissues surviving treatment were injured or changed in any way, except for the changes associated with cure of yellows. Some of the cured buds that survived treatment in each experiment were forced into growth by cutting back the trees into which they were transplanted. The shoots produced were normal in every respect.

HEAT TREATMENTS FOR THE CURE OF ROSETTE, LITTLE PEACH, AND RED SUTURE

The fact that peach trees affected by rosette may be cured by exposure to high temperatures has already been reported (10). In the course of work on treatment of trees for yellows, a number of experiments designed to test the possibility of curing little-peach and red-suture diseases were made. Both diseases yielded to treatments that proved effective in the cure of yellows. The results obtained with little peach in experiments employing the third method are summarized in table 4. Little-peach virus was inactivated by exposing buds for 115 minutes at 40° C., for 20 minutes at 46° C., for 8 minutes at 48° C., and for 3 minutes at 50° C. Somewhat erratic results were obtained with sticks exposed at 40° C. and at 42° C. The bud held at 40° C. for 105 minutes failed to transmit little peach, while that exposed at the same temperature for 110 minutes transmitted the dis-

TABLE 4.—*Thermal inactivation points of little-peach virus in bud sticks*

40° C.		42° C.		44° C.		46° C.		48° C.		50° C.		52° C.		54° C.		56° C.	
Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect
70	+	15	+	5	+	5	+	2	+	1	+	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$	+
75	+	18	+	10	+	10	+	4	+	2	0	1	0	1	0	$\frac{1}{2}$	0
80	+	21	+	15	+	15	+	6	+	3	0	$1\frac{1}{2}$	0	$1\frac{1}{2}$	0	$\frac{3}{4}$	0
85	+	24	+	20	+	20	0	8	0	4	0	2	0	2	0	1	0
90	+	27	+	25	+			10	0	5	0	$2\frac{1}{2}$	0	$2\frac{1}{2}$	0	$1\frac{1}{2}$	0
95	+	30	+					12	0	6	0	3	0	3	0	$1\frac{3}{4}$	0
100	+	33	+					14	0	8	0	$3\frac{1}{2}$	0	$3\frac{1}{2}$	0	2	0
105	0	36	0							10	0						
110	+	39	+							12	0						
115	0	42	0							14	0						
120	0	45	+							16	0						
		48	0														

+ indicates that bud transmitted disease.

0 indicates that bud did not transmit disease.

ease. Likewise, buds exposed at 42° C. for 36- and 42-minute intervals failed to transmit little peach, while those exposed for 39- and 45-minute intervals transmitted the disease. On the whole, the data recorded in table 4 indicate that little-peach virus is somewhat less resistant to heat than yellows virus. Tests with the 2 viruses at 48° C. were carried out at the same time and in exactly the same manner. Table 2 shows that yellows buds exposed for 8-, 10-, and 12-minute intervals all transmitted disease, and table 4 that little-peach buds exposed for the same periods of time all failed to transmit disease. Treatments for $\frac{1}{2}$ - and 1-minute intervals at 52° C. and 54° C., respectively, failed to cure yellows buds, while $\frac{1}{2}$ -minute treatments at these temperatures were sufficient to cure little-peach buds. At 56° C.,

TABLE 5.—*Thermal inactivation points of rosette and red-suture viruses in bud sticks*

Rosette							Red suture	
Temperature 40° C.		Temperature 50° C.					Temperature 50° C.	
Time in minutes	Effect	Time in minutes	Effect	Time in minutes	Effect	Effect	Time in minutes	Effect
50	+	1	+	2	+	+	1	+
55	+	2	+	4	+	+	2	+
60	+	3	+	6	+	+	3	0
65	+	4	+	8	+	0	4	0
70	+	5	+	10	0	0	5	0
75	+	6	+	12	0	0	6	0
80	+	8	0	14	0	0	8	0
90	+	10	0	16	0	0	10	0
100	+	12	0	18	0	0	12	0
110	+	14	0	20	0	0	14	0
120	+	16	0				16	0

+ indicates that bud transmitted disease.

0 indicates that bud did not transmit disease.

on the other hand, yellows virus was destroyed by a $\frac{1}{4}$ -minute exposure, while little-peach virus was not.

The results obtained in an experiment with red-suture virus are recorded in table 5. Buds exposed for 3 minutes or longer at 50° C. were cured. Those exposed for 1- and 2-minute intervals were not cured. Results from 4 experiments with bud sticks affected by rosette are also shown in table 5. These experiments were carried out in exactly the same manner as were those reported in tables 2 and 4. Rosette virus was not destroyed in buds exposed at 40° C. for as long as 2 hours. At 50° C. it was destroyed by an 8-minute treatment but not by a 6-minute treatment in 2 experiments, and by a

10-minute treatment but not by an 8-minute treatment in another experiment. The data indicate that rosette virus is more resistant to heat treatment than are the viruses of the other 3 diseases. This conclusion was confirmed by several hot-room experiments, one of which will be described.

On February 6, 1934, 6 healthy young trees about 3 feet high were inoculated with rosette virus by inserting a bud in the stem of each tree at a point about 15 inches above the soil level. At the same time, 6 other trees were similarly inoculated, but with yellows buds. Six additional trees were left uninoculated to serve as controls. One week after the inoculations had been made, 3 trees in each set were transferred to the hot room where they were incubated for 2 weeks. At the end of this period the trees were returned to the greenhouse from which they had been taken. When observations were made, 100 days after treatment, 2 of the 3 treated rosette trees showed symptoms of rosette, while the other tree appeared healthy. All of the 3 trees inoculated with rosette and not treated showed marked symptoms of disease. The 3 that were inoculated with yellows and then treated appeared healthy, while the 3 inoculated with yellows and not treated were badly diseased. All of the noninoculated controls were healthy. No changes appeared in the trees of this experiment up to the time it was ended, a year later. Since all of the yellows trees and only 1 of the rosette trees were cured by the treatment, the experiment brings evidence that rosette virus is more resistant to heat than yellows virus.

It was never possible to cure trees of rosette after they had become diseased throughout. Such trees are very susceptible to injury by heat, and, even when exposed at the low temperature of the hot room, they died during or shortly after treatment. Rosette trees were readily cured when treated within from 1 to 3 weeks after infection.

DISCUSSION

Yellows, rosette, little peach, and red suture are the only well-recognized virus diseases of plants that have been cured by heat treatments, but two other plant diseases suspected of belonging in the virus group have been so cured. When sugar-cane cuttings affected by the sereh disease of Java are immersed in water held at about 52° C. for 30 minutes to 1 hour, they are usually cured (6). The chlorotic-streak disease (12) affecting sugar cane in Hawaii, in Queensland (2), and in Java where it is known as the "fourth disease" has also been cured by a similar treatment at 52° C. for 20 minutes (13). So far as the writer is aware, neither of these diseases has been transmitted experimentally, and neither is definitely known to be caused by a virus. Wilbrink (15) concluded that sereh is probably due to an organism, since mosaic and similar diseases stand higher temperatures. Sugar-cane mosaic, which is transmitted by both mechanical and insect inoculations and

is definitely classified as a virus disease, has not been cured by heat treatments (4). Johnson (8) was unable to cure potato tubers of mosaic disease by heat. Blodgett (3) reports that heat treatments will not cure them of either mosaic or leaf roll, and Atanasoff (1) that it is evidently impossible to destroy the virus of stipple streak by heat treatments that do not injure tubers. Reddick and Stewart (14) and Fajardo (5) found that, when bean seeds affected by bean mosaic survived heat treatments, the virus also survived.

Finding that the 4 peach virus diseases under consideration yield to heat treatments, whereas several other virus diseases do not, suggests the possibility of a relationship between these diseases. Evidence indicating that yellows and little peach are closely related, but that rosette is distinct from both, has already been published (11). The fact that rosette virus is more resistant to heat treatments than virus of either little peach or yellows is in agreement with this evidence.

It is remarkable that yellows can be cured by a temperature below 34.5° C., and it would be interesting to know the minimum inactivation temperature. However, as temperature is lowered, the exposure period necessary for cure is greatly lengthened. This leads to practical difficulties that have not yet been overcome. Diseased trees held in the hot room for more than about a month become somewhat etiolated because of insufficient light. Such trees frequently die after they are returned to the greenhouse. Buds from sticks incubated in a water bath for long periods of time are apparently weakened, for many of them die after transplantation. On account of these difficulties, the minimum temperature of inactivation of yellows virus was not determined.

It has been suggested that viruses may be similar to the theoretical bearers of hereditary traits, which are known as genes. The experiments recorded here bring no evidence against this view, but they do show that the peach viruses that are inactivated by temperatures that cause no injury to peach tissues are less heat-stable than the genes of the peach, for the latter are apparently unaffected by exposures that readily inactivate virus.

A temperature of 35° C. (95° F.) is not unusual for the eastern United States during summer months, but such a temperature does not prevail over long periods of time except in the Southern States. This fact may afford a possible explanation for the southern limit of distribution of yellows in the Eastern States. It has long been known that at low elevations this limit lies only a short distance south of Washington, D. C., but extends far south of this latitude at high elevations. It thus follows an isotherm that passes across the eastern portion of southern Maryland and extends far to the south as it approaches the mountainous regions of Virginia and West Virginia, where summer temperatures are comparatively low. It is believed that the

temperatures that prevail south of this isotherm may be high enough and prolonged enough to inactivate yellows virus. Yellows occurs in the mountains of North Carolina and Georgia, but has never been reported from the lowlands of either State. Affected trees have doubtless been shipped into these regions.

Some of the reported experiments show the difficulty encountered in destroying yellows virus in the roots of potted trees. It was found to be much easier to cure tops than roots. This is presumed to have been due to cooling of roots by evaporation of water from the moist soil in which they were imbedded, rather than to any difference in resistance of virus in roots and in tops. The distribution of yellows virus in treated trees having healthy tops and diseased roots is strikingly like that of phony disease of peach. According to Hutchins (7), the phony virus is confined to roots. Heat-treatment experiments with yellows suggest that phony virus is absent from tops because of the high summer temperatures to which aboveground portions of orchard trees are exposed in Georgia where phony disease was studied.

No effort has yet been made to determine whether or not heat treatments will be useful in the control of virus diseases of peach. Orchard trees invaded by yellows virus frequently show no symptoms of disease until the end of the second season following infection. The danger of spreading yellows through the use of contaminated propagating material by nurseries has long been recognized. Valuable peach varieties developed in the eastern United States have not been introduced into California and other western states because of this danger. The evidence presented indicates that treated bud sticks could be used to introduce such varieties without risk of spreading the diseases under consideration.

Heat treatments for the cure of aerial portions of orchard trees affected by yellows, little peach, and red suture may prove practicable. If a diseased tree were covered by a canvas tent such as is used in fumigating, the air surrounding it might be raised, by means of an appropriate heating device, to a temperature high enough to cure the exposed top. The health of the tree might be sufficiently improved by destruction of virus in its top to justify the expense of treatment, especially if a 10- or 15-minute treatment proved effective. Such a method of applying heat would not destroy virus in roots, and it is not known whether a healthy top on a diseased root system would yield normal fruit. Moreover, the virus in the roots would spread into the top after a year or two and the treatment would then have to be repeated. It is not believed that such a method of treatment would prove successful for the cure of rosette, because of resistance of rosette virus to heat inactivation and the susceptibility of rosette trees to heat injury.

SUMMARY

Peach yellows was cured by incubating potted trees in a hot room held at a temperature that varied from 34.4° C. to 36.3° C. Cured trees assumed a normal habit of growth and produced healthy-appearing stems and leaves. The period of treatment necessary to effect a cure was longer for large than for small trees. Yellows virus was destroyed more quickly in small stems than in large ones and more quickly in tops than in roots that were imbedded in moist soil. Intermittent treatments did not prove effective. Dormant trees were cured by immersing them for about 10 minutes in a tank of water held at 50° C. The trees were not seriously injured by these treatments.

Yellows virus in buds was inactivated by immersing bud sticks in water held at a number of different temperatures. At 34.35° C. it was inactivated in from 4 to 5 days, at 38° C. in 11 hours, at 42° C. in 40 minutes, at 46° C. in 15 minutes, at 48° C. in 14 minutes, at 50° C. in from 3 to 4 minutes, at 54° C. in about 1 minute and 30 seconds, and at 56° C. in 15 seconds. The bud tissues were able to endure much longer treatments than were necessary to destroy virus.

Little peach, red suture, and rosette also were cured by exposure to heat. Little peach and red suture yielded to treatments that proved effective for the cure of yellows, but rosette was somewhat more refractory than the other three diseases.

FROM THE DEPARTMENT OF ANIMAL AND PLANT
PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR
MEDICAL RESEARCH, PRINCETON, NEW JERSEY

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SCLEROTIAL FORMATION IN RHIZOCTONIA SOLANI AS AFFECTED BY NUTRITIONAL AND OTHER FACTORS^{1,2}

W. B. ALLINGTON

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Rhizoctonia solani Kühn (*Corticium vagum* Burt) commonly overwinters in the sclerotial stage on potato tubers. Its attack on the young growing shoots and the cankering of the older stems causes more damage than do the sclerotia on the tubers. Nevertheless, rhizoctonia-infected tubers are decidedly inferior to noninfected ones either for seed or for table purposes.

Variations in the number and size of sclerotia on potatoes have been observed commonly. Since there is nothing in the literature that adequately explains these variations, the present work was undertaken in an effort to determine the factors involved in the formation of sclerotia.

Matsumoto (6) noted that *Rhizoctonia solani* formed more sclerotia with maltose than with cellulose and also more with casein than with potassium nitrate. He concluded, therefore, that the carbon and nitrogen sources were influential. He also observed that the hydrogen-ion concentration greatly affected sclerotial development in *R. solani*. He concluded that the effect was due mainly to the differences in availability of the nutrients at the different hydrogen-ion concentrations. Monteith and Dahl (7) report that the type and color of sclerotia fluctuate with different degrees of acidity.

Raeder, Hungerford, and Chapman (8) believe that the moisture content and character of the soil influence materially the number and size of sclerotia. Hurst (4) recommends digging potatoes as early as possible because he believes that low temperature and moisture stimulate the development of sclerotia on the tubers.

Conditions causing sclerotia to form in other fungi may be similar to those affecting their development in *Rhizoctonia solani*. Ezekiel and co-workers (2) report more sclerotia with *Phymatotrichum omnivorum* (Shear) Duggar under conditions best suited for rapid and heavy vegetative growth.

It is improbable that staling processes lead to sclerotial development. Neither *Phymatotrichum omnivorum* (5) nor *Rhizoctonia solani* (1) pro-

¹ The results presented in this paper formed part of a thesis submitted by the author to the Graduate Faculty of the University of Nebraska in partial fulfillment of the requirements for the degree of Master of Arts. June 1935.

² The writer wishes to acknowledge his indebtedness to Dr. R. W. Goss for advice and criticism during the course of the investigation and in the preparation of the manuscript.

duce a staling effect in the medium, yet both organisms produce sclerotia abundantly.

Experimental evidence in support of these viewpoints is rather meager. The best explanations probably are those involving nutritive relationships.

EXPERIMENTAL METHODS AND MATERIALS

The culture of *Rhizoctonia solani* used in these studies was a subculture of a single hyphal-tip isolate obtained from a germinating sclerotium removed from a potato grown in western Nebraska. The type of inoculum used in the culture was agar disks of a uniform size removed from the margin of a giant colony.

The media used were potato-dextrose agar and the following modification of Czapek's medium: magnesium sulphate 1.0 gm., potassium chloride 1.0 gm., potassium phosphate (di-hydrogen-) 2.26 gm., glucose 30.0 gm., urea 1.41 gm., agar 15.0 gm., water 1000.0 gm. The H-ion concentration, except when otherwise indicated, was adjusted to pH 6.2 after sterilization. Although filter-paper cones and sand with nutrient solutions were tried, the agar culture medium in Petri dishes proved most satisfactory and was used in all the experiments. Except where otherwise indicated, all cultures were incubated at 23° to 25° C.

THE EFFECT OF THE POTATO TUBER ON SCLEROTIAL FORMATION

Small moist chambers, approximately 8.5 cm. in diameter and 4 cm. in height, and containing 75 cc. of 1.5 per cent water-agar, were used for the cultures. Just before the agar solidified, small potatoes were sterilized in alcohol and one was placed into each culture dish near one side. The potatoes were large enough to allow about half their surfaces to be exposed above the agar. After the agar solidified, it was inoculated with a disk from the margin of the potato-dextrose-agar giant culture on the side opposite the potato. The check consisted of 10 moist chambers, which were treated similarly, without the addition of the potatoes.

The organism grew equally in all the cultures until the hyphae were within 5 to 10 mm. of the potato. At this time a temporary inhibitory effect by the potato was noted. The mycelium surrounded the potato without coming closer than 5 mm. to it before the opposite side of the dish had been reached, then the hyphae advanced to the surface of the potato and in two days sclerotia formed on it or near by on the agar surface. In the check cultures a few sclerotia appeared on and near the point of inoculation only.

The experiment was performed again with like results. Each time 10 cultures with each treatment were employed. When the synthetic agar medium was used as the inoculum carrier, the results were similar except in

the checks. Here, the sclerotia did not form quite so close to the point of inoculation as when potato-dextrose agar was used and they were fewer and were deeply submerged in the agar.

A similar set of cultures was grown using both potato-dextrose and synthetic agar for inoculation disks but the tubers were removed from the cultures before contact with the fungus. Although this experiment was not repeated, the results were very consistent in the 10 cultures employed.

The sclerotia invariably appeared near the source of inoculum in all the cultures. When potato-dextrose agar was used for the inoculum carrier, they appeared closer to the inoculation disk than when synthetic agar was used.

From these results it is obvious that the potato tuber influences the formation of sclerotia. Although these experiments show an inhibitive effect by the potato upon the fungus, it is improbable that this or any toxic effect from the potato stimulates sclerotial development. If some inhibitory agent were present that would cause sclerotia to form, they would have formed also near the point of inoculation as in the checks. An inhibitory substance did not remain in the agar after the removal of the potato, as shown by the unaffected progress of growth through this area.

The medium used in the cultures consisted of distilled water and agar only, leaving two isolated regions where added nutrients were available, namely, the inoculation disk and the potato. Since these regions were the seat of sclerotial formation, it appears that nutrition was a factor concerned with sclerotial development. It was interesting to note that the hyphae had to come in contact with the tuber if the latter was to affect sclerotial formation. It is possible, therefore, that the removal of food substances from the potato facilitates sclerotial development.

In a series of greenhouse experiments involving temperature studies, sclerotia were never observed to form upon buried foreign objects, such as smooth or frosted glass or porous clay surfaces. Many dormant potatoes were heavily covered with sclerotia while buried in the same soil. It appears, then, that the potato does more than merely furnish a surface where sclerotia may form.

THE EFFECT OF CARBON AND NITROGEN UPON

THE FORMATION OF SCLEROTIA

Source of Carbon

The effect of different kinds of carbon sources was studied by means of Petri-dish cultures on agar. The carbon compounds substituted for glucose in the synthetic medium were sucrose, potato starch, glycerol, and lactic acid. Equivalent amounts of carbon to that in the original synthetic medium were used. Two different nitrogen sources were employed, *i.e.*, for inorganic,

ammonium nitrate; for organic, urea. The nitrogen compounds were used in concentrations equivalent to the nitrogen in the synthetic medium.

The experiment was repeated 3 times and with like results. The differences in growth rate and sclerotial formation are shown in table 1. The

TABLE 1.—*Relative growth of mycelium and formation of sclerotia by Rhizotonia solani with different sources of carbon and nitrogen*

Compounds furnishing carbon ^a	Ammonia nitrate		Urea	
	Diameter of colonies after 3 days	Sclerotial formation after 10 days	Diameter of colonies after 3 days	Sclerotial formation after 10 days
	<i>mm.</i>		<i>mm.</i>	
Glucose	58	Abundant ^b	23	Medium
Sucrose	55	Abundant	40	Medium
Potato starch	60	Abundant	30	Medium
Glycerol	50	Medium	25	Slight
Lactic acid	50	None	15	None

^a The amount of carbon in each of the compounds was equivalent to the amount in the original synthetic medium, p. 832.

^b The word "abundant" refers to both size and number of sclerotia.

results shown in this table were taken from a representative set of cultures in a series of replications. The data show that the organism can use glucose, sucrose, and potato starch very readily for sclerotial formation. Glycerol and lactic acid are definitely less favorable. A marked difference is shown between the organic and inorganic nitrogen. Growth and the formation of sclerotia were much greater with ammonium nitrate. Sclerotia appeared earliest in those cultures that ultimately had the most sclerotia.

Amount of Carbon.—The effect of different amounts of carbon was studied with glucose as the carbon source. It was found in preliminary work that the transfer of mycelium to a starvation medium resulted in a very poor initiation of growth. Therefore, a different procedure was adopted in the preparation of the cultures. The various media, which were dilutions of the original synthetic medium with the same per cent of agar present, were pipetted into Petri dishes in equal amounts. After solidification, round disks 40 mm. in diameter were aseptically removed from the center of each dish. The resulting vacant areas were subsequently filled with a small amount of the synthetic medium and then inoculated. Any differences resulting from unequal growth initiation were greatly reduced before the hyphae reached the test medium.

The experiment was repeated, involving many cultures that gave consistent results. Table 2 shows the average growth and sclerotial formation of one series of cultures. Diffusion of the carbohydrate from the central

TABLE 2.—*Relative total growth and sclerotia formation by Rhizoctonia solani with decreasing amounts of carbohydrate, nitrogen, and total nutrients*

Concentration of variant		Average diameter of colonies after 3 days	Sclerotial develop- ment after 10 days
	<i>Per cent</i>	<i>mm.</i>	
Glucose	3.00	66	Very slight
“	1.50	67	Slight
“	0.75	65	Medium
“	0.35	69	Medium
“	0.20	69	Abundant ^a
“	0.00	70	Abundant
Urea	0.140	75	Slight
“	0.070	68	Slight
“	0.030	69	Slight
“	0.015	66	Very slight
“	0.007	66	Very slight
“	0.000	62	None
Total Nutrients	100.0	73	Very slight
“ “	50.0	74	Medium
“ “	25.0	75	Slight
“ “	12.0	69	None
“ “	6.0	70	None
“ “	0.0	65	None

^a The word “abundant” refers to both size and number of sclerotia.

portion of the culture undoubtedly took place. Nevertheless, the effect of smaller amounts of carbon was clearly demonstrated. There were no significant differences in the growth response of the mycelium within these small culture dishes with their varying amounts of carbohydrate, but the number of sclerotia greatly increased when the carbohydrate was diminished. The cultures having the most sclerotia also were those in which they formed first.

The sclerotia never appeared in these cultures until the entire volume of agar in the dish had been covered with fungous hyphae. In an attempt to eliminate this condition, large moist chambers 17 cm. in diameter were later used for culture dishes. Even in these giant culture dishes, regardless of the nutrient conditions, sclerotia never appeared before all the agar was covered.

Interesting results were obtained when varying amounts of glucose were added to the medium in the large moist chambers. A portion of the basic synthetic medium was substituted in the center of the moist chambers containing the test media, the same as with the above mentioned Petri-dish cultures. When the hyphae reached the periphery of the moist chambers,

sclerotia soon appeared. The position of these sclerotia was particularly interesting. In all the cultures containing glucose with a concentration of 0.35 per cent or more, the sclerotia formed upon the edge of the moist chamber or very close to it. In the cultures containing 0.20 per cent, and in those with no glucose, the sclerotia formed in a ring $\frac{3}{4}$ and $\frac{1}{2}$ the way out, respectively, from the center of the dish.

Carbohydrate concentration is thereby shown to affect sclerotial formation when the nitrogen and other nutrients are constant. There is yet no means of differentiating between the effect of total concentration of carbohydrate and the relationship between carbohydrate and nitrogen. Subsequent experiments deal in part with this phase of the problem.

Source of Nitrogen

The following experiment was conducted to determine any further relationships between the source of nitrogen and the development of sclerotia.

The basic synthetic medium was used with the various nitrogen compounds substituted for urea. The concentration of the nitrogen compounds was determined by using amounts of nitrogen equivalent to that in the basic synthetic medium. These nitrogenous compounds were: urea, asparagine, calcium nitrate, ammonium nitrate, and sodium nitrate. The test medium was pipetted in equal amounts into Petri dishes. All inoculations were made directly upon this medium at the center of each dish.

Variation in growth rate was insignificant with the different sources of nitrogen tested. On the other hand, sclerotial formation was greatly influenced by the kind of nitrogen (Fig. 1, A). There was variation not only in numbers but in size and character of the individual sclerotia. The cultures having the most sclerotia also were the first to have them appear.

The use of urea resulted in the greatest number of sclerotia; but they were very small, and the majority were submerged in the agar. Close examination of the cultures (Fig. 1, A) disclosed innumerable minute sclerotia in the dish containing urea. That containing asparagine showed several large black sclerotia, accompanied by a great many smaller ones. Many of the smallest were submerged and were similar to those in the dish containing urea. The sclerotia in the dish containing calcium nitrate were all upon the surface of the agar, but the majority were exceedingly small. They tended to coalesce, forming large irregular black blotches upon the agar surface. In the dish containing ammonium nitrate there were fewer sclerotia, most of them were superficial, of medium size, and typically round and hard. The sclerotia in the culture containing sodium nitrate were mostly small and comparatively few.

After sclerotial formation the medium was removed from the culture dishes, melted and the pH was determined. The results shown in table 3

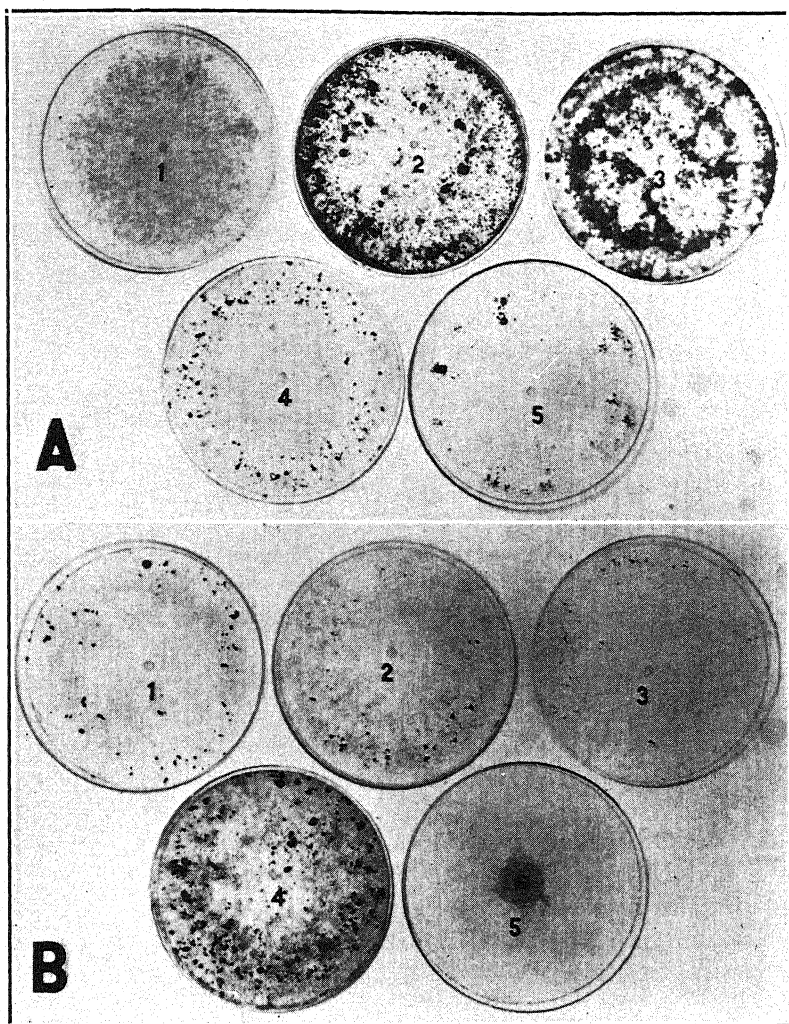


FIG. 1. Cultures of *Rhizoctonia solani*. A. Differences in sclerotial formation with various sources of nitrogen: 1, Urea; 2, asparagine; 3, calcium nitrate; 4, ammonium nitrate; 5, sodium nitrate. B. Differences with various H-ion concentrations. 1, pH 4.0; 2, pH 5.0; 3, pH 6.0; 4, pH 7.0; 5, pH 8.0.

clearly indicate that *Rhizoctonia solani* is able to change the pH of its substratum. The direction and extent of change is dependent upon the nitrogen source.

The results obtained by using different nitrogen sources probably cannot be explained by the effect of pH. Sodium nitrate and calcium nitrate cause the pH to rise to somewhat similar values, yet sclerotial formation

TABLE 3.—*Changes of H-ion concentration of the medium by Rhizoctonia solani with different sources of nitrogen*

Nitrogen sources ^a	Final pH ^b
	<i>pH</i>
Urea	6.1
Asparagine	6.0
Calcium nitrate	6.8
Ammonium nitrate	4.2
Sodium nitrate	6.9

^a The concentration of nitrogen used in all compounds was equivalent to that in the basic synthetic medium.

^b The initial pH of all media was 6.2.

is not similar with the two compounds. Ammonium nitrate and sodium nitrate, both, yield sclerotia sparingly, yet the former leads to an extremely low pH and the latter to a higher pH.

Concentration of Nitrogen.—The following experiment shows the relationship between the amount of nitrogen present and sclerotial development.

The synthetic medium, combined with the method previously described of surrounding the full nutrient medium with the test media in Petri dishes, was used. The test media consisted of the synthetic medium with varying amounts of urea substituted as the single nitrogen source. Equal amounts of the media were pipetted into the culture dishes to insure comparable growing conditions. The experiment was repeated once with consistent results.

The results of the experiment are shown in table 4. Growth rate and sclerotial formation were decreased simultaneously when the nitrogen was decreased. When nitrogen was absent from the medium there was no sclerotial formation. The results are the reverse of those obtained with decreasing carbohydrates, as shown in table 2.

THE EFFECT OF A DECREASE IN TOTAL NUTRIENTS UPON SCLEROTIAL FORMATION

A decrease in carbohydrate has been shown to stimulate sclerotial development, while a decrease in nitrogen inhibits sclerotial formation. It was then necessary to determine how the organism reacts when all the nutrients were decreased.

The usual type of Petri dish culture was used, *i.e.*, the test medium surrounded the usual basic synthetic medium. A large quantity of the basic synthetic medium was made and diluted in distilled water to give the following percentages of the original concentration: 100, 50, 25, 12, and 6 per cent. One series of plates contained no added nutrients in the test medium. Inocu-

lation was made as usual upon the medium in the center of the dish, and the mycelium grew out over the test medium. The experiment was repeated once, with 5 cultures for each test medium.

The results, which clearly indicate the effect of decreasing nutrients, are shown in table 2. In 10 days sclerotia had not appeared at concentrations below 25 per cent. Evidently, sclerotial formation is not a simple starvation phenomenon. Previous tests have given more sclerotia with low carbohydrate or high nitrogen. It will be observed in table 2 that sclerotial formation first increased with lower nutrients and then decreased sharply. The increase with the initial reduction in nutrients of 50 per cent was probably due to the effect of decreasing the carbohydrate. The latter decrease in sclerotial development was the effect of lower nitrogen.

THE EFFECT ON SCLEROTIAL DEVELOPMENT OF ADDING NUTRIENTS TO STARVED CULTURES

After determining that sclerotial development was affected by nutritional factors, it was decided to add certain nutrients to starved cultures and note the reactions of the fungus.

A starvation medium consisting of 1.5 per cent agar in distilled water was used in large moist chambers, 5 cm. deep and 17 cm. in diameter. A small disk of the basic synthetic medium was placed in the center of each culture, in place of the starvation agar, and inoculated. Preliminary experiments with the same type of cultures showed that, by the use of a small disk of synthetic medium in the center, the organism would grow out over the starvation medium but that it never formed sclerotia. At the time of inoculation, the test substances were added near the periphery of the moist chamber. The following were placed in the culture dishes: sterile potatoes, aseptic potato disks, glucose in agar, basic synthetic agar, ammonium nitrate in agar, urea in agar, and a check composed of distilled water and agar only. When the nutrient compounds were added to an agar medium, the concentrations were the same as in the basic synthetic medium. The aseptic potato disks were taken from mature potatoes within one centimeter of the periderm. The agar and potato disks were 12 mm. wide and 3 mm. thick. The sclerotia were abundant around the potato disk, but occurred very sparsely around the disk containing urea. In the other cultures sclerotia formed on the potato and around the ammonium nitrate disk. A decided stimulation to mycelial growth was evident with all the nitrogen compounds.

The cause of sclerotial development is not simple starvation. Unless the fungus colony could grow over the entire agar surface before the food supply became sufficiently exhausted to stop growth, the sclerotia failed to appear. In this experiment the food supply was exhausted to that extent and the value of certain substances in the formation of sclerotia was demonstrated. The results show conclusively that high carbohydrate is of no

effect but that the addition of nitrogen has a decided stimulatory effect upon sclerotial development. The results conform very favorably to those of previous experiments, which showed that high nitrogen and low carbohydrate gave the most sclerotia.

THE EFFECT OF THE H-ION CONCENTRATION OF THE MEDIUM ON SCLEROTIAL FORMATION

It has been shown that the H-ion concentration of a medium is sometimes changed during the growth of *Rhizoctonia solani* (3). The following experiment was conducted to determine the effect of different initial H-ion concentrations upon sclerotial development.

The synthetic medium was adjusted to the desired pH value by the addition of HCl or NaOH. The following pH values were employed: 4.0, 5.0, 6.0, 7.0, and 8.0. The medium was pipetted into Petri dishes and inoculated.

The results are shown in figure 1, B. *Rhizoctonia solani* was able to grow at every pH value tested, although the growth rate was extremely low at a pH 8.0. Maximum growth occurred at pH 7.0 and sclerotia were formed in abundance. Fewer sclerotia developed at 4.0, 5.0, and 6.0 and there was very little difference in these sets. At pH 8.0 the mycelium presented a peculiar appearance. The hyphae were densely matted together, but sclerotial hyphae were not observed.

Growth and sclerotial development of *Rhizoctonia solani* were favored by neutral or acid conditions. Very little growth was possible at pH values above 7.0.

THE EFFECT OF TEMPERATURE ON SCLEROTIAL FORMATION

It had been suggested (4) that low temperature may cause *Rhizoctonia solani* to form sclerotia on the potato tuber in the fall. If low temperature affects sclerotial formation in the soil, it is probable that a similar reaction would occur in pure culture and the following experiments were outlined to determine these two points.

Effect in Pure Culture

Petri dishes containing potato-dextrose agar were inoculated and incubated at 25° C. for one day. At this time the colonies were approximately 20 mm. in diameter. Five cultures were then placed in incubators at each of the following temperatures: 5°, 10°, 15°, 20°, and 25° C. Growth measurements were taken daily and the time and character of sclerotial formation were carefully noted.

The rate of growth was considerably less at lower temperatures as reported by Monteith and Dahl (7). At 25° C., the highest temperature

to which the cultures were exposed, the growth was the greatest. At 5° the organism was unable to grow, but remained alive during the exposure.

Sclerotia appeared first in the cultures subjected to the higher temperatures, probably due to the fact, previously stated, that they do not form until the medium is covered with mycelium.

When cultures held at 5° C. for 10 days were transferred to a temperature of 25° C., normal growth was resumed and continued until the culture dish was covered, then sclerotia formed predominately at the center of the dish. This region was precisely the same as that covered by mycelium during exposure to low temperature. When cultures were held at 5° for 20 days, no sclerotia appeared until exposure to higher temperatures. Microscopic examination of the cultures at the time of transfer from 5° to 25° revealed no tendency toward sclerotial formation.

The peculiar clustering of sclerotia in the central region of each culture probably resulted from different metabolic activity of the fungus while at low temperature an activity that caused different nutritive conditions to prevail.

Effect in Soil

Greenhouse soil was inoculated with a heavy suspension of *Rhizoctonia solani* mycelium and then mixed well before potting. Sclerotia-free potatoes were disinfected with alcohol and buried for 20 days in the soil which was kept at an optimum moisture with both high and low temperature. At the termination of the experiment the potatoes were dug, washed, and graded for the presence of sclerotia.

The data obtained were inconclusive and further repetition of the experiment is required. However, it was of interest to note that sclerotia formed under these conditions. In some cases very heavy infection was obtained. It is possible, therefore, that potato seed-pieces, which are healthy when planted, may have abundant sclerotia on them if removed from the soil 20 days later for examination.

DISCUSSION

The results indicate that a number of factors influence sclerotial development in *Rhizoctonia solani*. These factors may act collectively or individually to enhance or to inhibit the effect of other factors.

Sclerotia did not form in Petri-dish cultures until the growth of the organism was physically limited. This phenomenon occurred regardless of whether dishes 10 cm. or 17 cm. in diameter were used. Apparently, therefore, the age of the cultures was of little significance in sclerotial formation.

Sclerotial formation is affected greatly by differences in nitrogen source. Although the extent and direction of the change of H-ion concentration in

the medium is influenced by the nitrogen source, the variations of sclerotial development cannot be explained on this basis. It is improbable that the changes in H-ion concentration in the medium affect the solubilities of the nitrogenous compounds sufficiently to account for the differences in sclerotial development.

Rhizoctonia solani can readily utilize glucose, sucrose, and potato starch. These substances sustained good growth and sclerotial development with no detectable differences. Matsumoto (6) has found the organism capable of digesting cellulose and other complex carbohydrates.

Sclerotial formation decreased when less than 50 per cent of the original amount of nutrients were added to the culture medium. When slight reductions were made, however, sclerotial development increased. After the agar is completely covered mycelial growth is slowed up considerably. At this time the assimilation of nitrogen is at a minimum, while the assimilation of carbohydrate is still taking place at a relatively high rate. The demand for carbohydrate is greater than for nitrogen, hence the decrease in carbohydrate is the first to have an effect. The increase in sclerotial development due to high nitrogen can be explained on the same basis. Cultures with high nitrogen invariably produce a heavy mycelial growth and demand more carbohydrates for maintenance.

It has been found that greater sclerotial infection occurs on potatoes in western Nebraska that were planted early and matured early during periods of high temperatures.³ This, along with sclerotial development upon mature tubers late in the fall, indicates a correlation between sclerotial formation and maturation of the potato or with higher temperature conditions. Werner (9) has analyzed potato tubers at different stages of maturity and reports an increase in the percentage of total nitrogen with maturation. This increase in nitrogen might cause an increase in sclerotial development just as it did in pure culture. Since pure-culture studies have shown more sclerotia at higher temperatures, the above mentioned results might be explained on that basis. The appearance of heavy infections in the fall, however, tends to indicate that maturity has more influence.

Since low temperature retards sclerotial formation in pure culture, it is doubtful if it hastens the process in nature. The conclusions of many who have observed the increased sclerotial infection upon potatoes when they are harvested late in the season, indicate that temperature is considered to be the causal factor. The host plant has never been considered in an explanation of this phenomenon. Since nutrition plays a part in sclerotial formation and the potato may be penetrated by the fungus, the host must be considered.

Sclerotia upon potatoes are more common near the stem end of the tubers. There are two possible explanations of this phenomenon. The

³ Unpublished data by R. W. Goss, Nebraska Agr. Expt. Sta.

periderm of the potato near the stem end is the oldest and has been exposed the longest to possible infection. This part of the potato is more mature and differs chemically from the other parts. Therefore, if maturity affects sclerotial development, a predominance of sclerotia might be expected near the stem end.

Growth and sclerotial development have been shown to be favored by a pH near 7.0. The number and size of sclerotia varied greatly at different pH values. In many cases sclerotial variations in different localities may be due to the soil reaction and other conditions and not to different strains of the organism.

A careful investigation of the effect of staling upon sclerotial development has not been made. However it has been shown by Allen and Haenseler (1) that *Rhizoctonia solani* does not produce substances in the medium that are autotoxic. Since the medium employed in this investigation was very similar to that used by Allen and Haenseler, the author has disregarded the staling factor. The cotton root-rot fungus produces sclerotia, yet has no staling effect upon its medium (5). It is very improbable, therefore, that staling is a factor in causing sclerotial development.

The effect of moisture upon the formation of sclerotia has not been investigated. Field observations are somewhat contradictory. An investigation of this phase of the problem is desirable.

If we are to determine the actual cause of sclerotial development, we must conceive of an intricate set of conditions, which, collectively, have an influence in one direction. The complexity of this mechanism appears at present to be very great.

SUMMARY

The effect of various nutritional factors, temperature, and H-ion concentration of the substratum upon sclerotial development of *Rhizoctonia solani* Kühn was studied in pure culture. Other factors, commonly attributed to be influential in sclerotial development, are discussed.

Sclerotia never formed before all the agar in the culture dish has been covered by the colony.

The potato tuber itself had an influence upon sclerotial formation. This influence may be enhanced by maturation due to the increased nitrogen content of the tuber at that time.

Sclerotia formed most readily with relatively low carbohydrate and high nitrogen.

Extreme variations in sclerotial development were evident with different nitrogen sources. The nitrogen compounds in the order of decreasing sclerotial formation were: calcium nitrate, asparagine, ammonium nitrate, urea, and sodium nitrate.

No differences in sclerotial development were observed with glucose, sucrose, or potato starch as the carbon source. Glycerol and lactic acid were not utilized readily by the fungus.

A small decrease in total nutrients caused increased sclerotial development; a further decrease resulted in decreasing sclerotia.

R. solani was able to change the H-ion concentration of its substratum, the direction and extent of change depending upon the nitrogen source. This does not account for the variations in sclerotial development with different nitrogen sources.

An H-ion concentration near 7.0 favored growth and sclerotial formation. The organism was quite tolerant to acid but not to alkaline conditions.

Temperature changes did not stimulate sclerotial development in pure culture.

In general, sclerotial development paralleled vegetative growth.

Sclerotia formed upon healthy seed potatoes buried in inoculated soil. It is possible, therefore, to have healthy seed-pieces become infected after planting.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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THE TOLERANCE OF ERYSIPIHE POLYGONI AND CERTAIN OTHER POWDERY MILDEWS TO LOW HUMIDITY¹

CECIL E. YARWOOD²

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METHODS

Conidia for germination and inoculation tests were taken from mildewed greenhouse plants during the light portion of the day, unless otherwise mentioned. To insure a more uniform distribution of the conidia on the test substrata, the slides, Petri dishes, leaves, or plants were placed in the bottom of a large can and conidia were dusted on them from above. Unless otherwise mentioned the dry slides with conidia were placed in Petri-dish moist chambers in the diffuse light of the laboratory for germination tests. Germination percentages always are based on counts of 200 or more conidia, including shrivelled ones. Shrivelled conidia, evidenced by wrinkled walls, loss of turgor and vacuolation, and decrease in size and transparency, were commonly present in small numbers in any sample of conidia from field or greenhouse plants, and they did not germinate. When subjected to low humidity many normal conidia became shrivelled either without germination or during germination. Hence, counts of shrivelled conidia include those that were shrivelled before being placed to germinate, as well as those shrivelled during the germination interval. When conidia were placed at 100 per cent relative humidity, the number of conidia shrivelled during germination would be negligible, while, at low humidity, the number of such conidia might be considerable.

To produce different relative humidities, solutions were prepared with C. P. sulphuric acid and distilled water; and these solutions are assumed to produce the relative humidities tabulated by Stevens³, except for the concentrated acid (S. G. 1.84), which was estimated from Stevens' figures to give a relative humidity of approximately 0. Dehydration of the air of the chambers and of the experimental leaves and spores weakened the sulphuric acid solutions. Consequently, the relative humidities did not remain at the theoretical values they had at the time the solutions were prepared. As the amount of acid was large in comparison with the amount

¹ Contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture; the Botany Department, Purdue University Agricultural Experiment Station; the Department of Plant Pathology, University of Wisconsin; and the Division of Plant Pathology, University of California.

² The writer acknowledges his indebtedness to Dr. M. W. Gardner for assistance during the course of the work and in the preparation of the manuscript.

³ Stevens, N. E. A method for studying the humidity relations of fungi in culture. *Phytopath.* 6: 428-432. 1916.

of experimental material, however, this source of error is not considered significant in these studies. The stillness of the air in the experimental humidity chambers might be expected to allow considerable local variation in relative humidity within the chambers and a higher humidity near the leaf and fungus surfaces. But, since, in two germination tests in which rapid air circulation within the chamber was provided, the results were similar to those where no artificial circulation was provided, local variations in the relative humidity within the chambers are not considered to be important in the small chambers.

The laboratory environment used extensively in these tests has not been controlled, but has remained fairly constant. Cultures were protected from direct sunlight, but were maintained in strong diffuse light near an east window. The temperatures in the laboratory during these tests have been observed to vary between the limits of 19° and 24° C. and numerous measurements of relative humidity during the day and night indicate that this has remained between the limits of 25 and 55 per cent, with an average of about 44 per cent.

THE EFFECT OF HUMIDITY ON THE GERMINATION OF CONIDIA

Results of 3 tests to measure the gross relation between humidity and the germination of conidia of clover, barley, oat, and sunflower mildews are given in table 1. Germination resulted when the conidia were on the surface of water, suspended in water, on dry glass at 100 per cent relative humidity, and on dry glass at about 0 relative humidity, and only with barley and sunflower mildew conidia at about 0 relative humidity was the amount of germination significantly lower than that under the best conditions provided in these limited tests. Relative humidities intermediate between 0 and 100 per cent have been extensively tested; but, as would be expected, it was difficult to measure differences in germination due to even large differences in relative humidity. As the objection has been raised that the circulation of air in the stationary Petri-dish germination chambers used in these tests might be inadequate to insure a thorough distribution of air at the relative humidity determined by the humidity-regulating solution, other tests were made in which the conidia were on the inner surface of Petri-dish lids, 15 cc. of humidity-regulating solution was placed in each dish, the dishes were sealed with rubber bands, and then placed on an inclined rotating table, so that the humidity-regulating solution was kept continuously agitated. Results in such chambers were similar to those in which no such precautions for humidity regulation were taken, and 47 and 87 per cent, respectively, germination of clover mildew conidia resulted over C. P. sulphuric acid in 2 successive tests in such chambers.

TABLE 1.—Percentage germination of conidia resulting under different conditions of humidity for 6 hours at about 22° C.

Location and organism	Conidia on the surface of water	Conidia at 100 per cent relative humidity on dry glass	Conidia suspended in water	Conidia at 0 relative humidity on dry glass
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Lafayette, Indiana, 1930 <i>Erysiphe polygoni</i> from red clover		56		26
Madison, Wisconsin, 1933 <i>E. polygoni</i> from red clover		76		47
<i>E. graminis</i> from barley ..		24		2
Berkeley, California, 1935 <i>E. polygoni</i> from red clover	65	86	57	63
<i>E. graminis</i> from oats	59	39	41	25
<i>E. cichoracearum</i> from sunflower	24	9	13	1.5

While conidia of clover mildew will germinate at low relative humidities, they may be killed by only a short period of drying under certain conditions. Conidia attached to conidiophores on mildewed, but otherwise normal, leaves retain their germinability for several days under field conditions; but, if the mildewed leaves are detached from the plant and allowed to dry out, the conidia soon are killed. A number of mildewed leaves were cut from field plants at Madison, Wisconsin, at 7 p. m., July 9, and hung in the field to dry. Spore mounts were made from these leaves at intervals thereafter, and the germinability of the conidia and the number of shrivelled conidia were determined. The results, given in table 2, indicate that most of the conidia were dead within 36 hours.

In certain cases the germinability of conidia may be favored by drying. Several tests with clover mildew have given unsatisfactory results, but the results of 3 tests with bean mildew appear conclusive and are given in table 3. Conidia for these tests were produced in the laboratory. For the flask cultures, greenhouse plants with 2 true leaves were excised above the soil level and the stems placed in flasks of tap water and the leaves held in the laboratory environment. Such excised plants formed roots in the water, regenerated new leaves and supported a luxuriant growth of mildew which showed a diurnal cycle⁴ characteristic of *Erysiphe polygoni*. The

⁴ Yarwood, C. E. The diurnal cycle of the powdery mildew *Erysiphe polygoni*. Jour. Agr. Res. 52: 645-657. 1936.

TABLE 2.—*Germination of conidia from mildewed clover leaves cut from field plants and dried in the field. 1933, Madison, Wisconsin*

Time when leaves were cut from plants	Time of preparing spore mount	Period during which excised leaves were exposed to field conditions	Germination interval in moist chambers at 25° C. in dark	Spores germinated	Spores shrivelled
		Hours	Hours	Per cent	Per cent
7 p. m. July 9	7 p. m. July 9	0	12	56	5
"	7 a. m. " 10	12	14	68	3
"	10 " " " "	15	12	50	4
"	1 p. m. " " "	18	10	22	8
"	7 " " " "	24	12	1.8	87
"	7 a. m. " 11	36	11	0.3	89

dish cultures referred to in table 3 were mildew-infected excised leaves on 5 per cent sucrose solution in Petri dishes. Hence, the conidia from the flask cultures were produced at about 40 per cent relative humidity, while those from the dish cultures were produced in a saturated atmosphere. Yet, the general results with conidia from these two sources were similar. In the manipulation of each of these tests, 4 slides were dusted at about noon with conidia from each spore source. One slide was placed immedi-

TABLE 3.—*The effect of relative humidity and drying on the germination of conidia of E. polygoni from bean leaves at about 22° C., Berkeley, California, 1935*

Treatment given the conidia	January 3		January 5		January 7	
	Dish culture germination	Flask culture germination	Dish culture germination	Flask culture germination	Dish culture germination	Flask culture germination
100 per cent R. H.	Per cent 9	Per cent 0	Per cent 13	Per cent 8	Per cent 21	Per cent 14
0 R. H. ^a	7	27	30	17	30	11
Dried 2 hours, then at 100 per cent R. H. ...	42	13	48	15	83	50
Dried 8 hours, then at 100 per cent R. H. ...	83	20	63	17	41	23

^a Most germinated and nongerminated conidia had shrivelled after 24 hours under these conditions.

ately in a Petri-dish moist chamber at 100 per cent relative humidity; one was placed at 0 relative humidity (in a Petri dish with C. P. sulphuric acid); one was allowed to dry for 2 hours on the laboratory table and then transferred to 100 per cent relative humidity; and another was allowed to dry for 8 hours on a laboratory table and transferred to 100 per cent relative humidity. Germination was counted on all slides at 24 hours after preparing the mounts. The results show that drying stimulated germination in most cases and that germination over sulphuric acid was greater than that over water, though most of the conidia over sulphuric acid for 24 hours were severely shrivelled. Conidia dried for 2 or 8 hours showed higher germination in most cases than those at 0 relative humidity continuously. Conidia produced in dish cultures at approximately 100 per cent relative humidity showed higher germination in these tests than did those produced in flask cultures at about 40 per cent relative humidity.

THE EFFECT OF TEMPERATURE ON THE TOLERANCE OF CONIDIA TO LOW HUMIDITY

Although low relative humidity does not always reduce the amount of germination, it always causes considerable shrivelling of the germinated and nongerminated conidia, and this desiccation action is more pronounced at high temperatures. The results of a test of the relation of temperature to desiccation injury of clover mildew and mustard mildew is given in table 4. In this test the percentage of shrivelled conidia in the mounts over sulphuric acid was progressively greater as the temperature was increased, until at 22° C. all the conidia were shrivelled in the 30-hr.

TABLE 4.—*The effect of temperature on the tolerance to low humidity of the conidia of E. polygoni from red clover and from mustard. March 1935, Berkeley, California^a*

Temperature of germination	Relative humidity in germination chamber	Clover mildew		Mustard mildew	
		Germination	Shrivelled conidia	Germination	Shrivelled conidia
°C.	Per cent	Per cent	Per cent	Per cent	Per cent
13	100	19	0	40	14
	0	37	20	0	48
16	100	37	0	45	9
	0	40	96	4	72
19	100	41	0	46	10
	0	47	95	3	95
22	100	63	0	41	13
	0	65	100	0.5	100

^a Conidia of clover mildew from greenhouse potted plants. Conidia of mustard mildew produced on excised leaves. Conidia placed in darkness and germination counted after 30 hours.

period of the test. The slightly greater germination of clover mildew conidia at the lower humidity at each temperature is of interest. The test also shows that conidia of clover mildew are more tolerant to low humidity than are the conidia of mustard mildew (also *Erysiphe polygoni*). A deduction from this and other tests not shown in the table is that conidia are more sensitive to the desiccative action of sulphuric acid after germination than before. For instance, in one test at 0 relative humidity for 24 hours, 80 per cent of the total conidia had shrivelled, 98 per cent of the germinated conidia had shrivelled, while 67 per cent of the nongerminated conidia had shrivelled.

THE ACTION OF HOST LEAVES IN PROTECTING CONIDIA FROM THE INJURY
CAUSED BY DESICCATION AND HIGH TEMPERATURE

As already indicated, while conidia may germinate on glass slides at the lowest relative humidities tested, they are soon shrivelled and killed under these conditions, especially at high temperatures. With conidia on host leaves this injurious action of low humidity is much less apparent, and continued luxuriant development of clover mildew from inoculation to sporulation has occurred at the low relative humidity produced in closed chambers by C. P. sulphuric acid. The protective action of the turgid leaf in reducing the injurious action of low humidity on the fungus was apparent also when the mildew colonies on leaves were subjected to drying at high temperatures in different ways. The results of one test given in table 5, show that when conidia are exposed on dry slides or on detached

TABLE 5.—The effect of exposure to 35° C. and 50 per cent relative humidity on the subsequent germination of clover mildew conidia on glass slides at 100 per cent relative humidity, 20° C. and in light for 8 hours

Period of exposure at 35° C. (minutes)	Conditions under which spores were exposed		
	detached spores on dry slides	Spores attached to mycelium on which they were produced	
		leaf pieces on dry glass plate	leaf pieces floating on water
	Per cent	Per cent	Per cent
0 (check)	33	29	49
5	47	21	56
20	80	58	51
80	0 ^a	0 ^a	72
320	0 ^b	0 ^b	32

^a Spores not visibly shrunk.

^b Spores shrunk.

pieces of leaves that can be easily dried out, they are soon killed, while, when attached to a living leaf kept alive on water, they are but slightly injured at this high temperature.

The effect of the host in protecting the conidia from the injurious action of combined low humidity and high temperature is very pronounced under field conditions. In studies of the diurnal cycle of the germinability of conidia of clover mildew at Madison, Wisconsin, in 1933 (4), glass slides were dusted with conidia from field plants and some of these slides were exposed to the field summer environment, including temperature, sunlight, and humidity. Conidia on such slides were all shrivelled and killed in as short a time as 3 hours during the hottest part of the day, but similar conidia dusted upon a living leaf in a similar environment gave rise to successful infection. Also, conidia attached to the conidiophores on infected leaves remain turgid for several days under the normal summer environment in the field and in the greenhouse. The action of the host in protecting the conidia from the injurious action of low humidity and high temperature under field conditions possibly is due to the lower temperature and higher humidity at the leaf surface.

THE CHANGE IN VOLUME OF CONIDIA DURING GERMINATION

Germination of the spores of most fungi requires free moisture and the germination process is characterized by intake of water and increase in volume of the spore. That this general rule does not apply to the conidia of certain powdery mildews already has been indicated, and further data were secured by measuring the changes in volume of conidia during the germination process. Conidia of *Erysiphe polygoni* from clover, *E. polygoni* from cabbage, and *E. graminis* from barley were dusted onto slides or cover glasses, and the length and diameter of individual conidia were measured at the time of preparation of the spore mounts, and after several hours' exposure to known relative humidities. Germinated and nongerminated conidia were tabulated separately.

As examples of spores believed to germinate normally in free moisture, the following were used: The urediospores of *Uromyces fallens* (Desm.) Kern from clover leaves and the conidia of *Sclerotinia fruticola* (Wint.) Rehm, *Collectotrichum trifolii* S. M. Bain, and *Cicinobolus cesatii* de Bary from agar cultures. The conidia of these 3 latter fungi have failed to germinate on dry glass slides but some germination of the urediospores of clover rust has resulted on apparently dry slides at 100 per cent relative humidity. As these spores were germinated in or on the surface of water, it was impracticable to follow the changes in size of the same spore during germination. Therefore, random spores in the hanging drops were

measured at the time of preparing the mount and after known incubation intervals.

Spore volume was calculated from the formulae given in table 6, which were supplied by M. A. Ingraham, Department of Mathematics, University of Wisconsin, on the basis of drawings supplied by the writer. Spore shape changed but slightly during germination, and the same formula for volume was used before and after germination.

The number of spores measured, germination interval, environmental conditions during germination and changes in volume of spores for each species tested, are given in table 6. The powdery mildew conidia decreased about 24 per cent in volume during germination, rust urediospores showed no change in size, and the conidia of the other fungi tested showed a pronounced increase in size during germination. All spores, whether germinated or not, remained turgid during the period of these tests. The volume changes of the nongerminated spores (not tabulated) was about the same as that of the germinated spores, *i.e.*, nongerminated conidia of *Erysiphe* decreased in volume, nongerminated rust spores remained the same size, and those of *Colletotrichum*, *Sclerotinia*, and *Cicinnobolus* increased in volume under these experimental conditions.

When conidia of *Erysiphe polygoni* from clover and from cabbage were floated on water or placed on dry slides at 100 per cent relative humidity, there was also a decrease in volume of the conidia during germination, but the decrease was not so large or so significant as at 80 per cent relative humidity.

THE EFFECT OF RELATIVE HUMIDITY ON THE GROWTH OF MYCELIUM OF CLOVER
POWDERY MILDEW. BERKELEY, CALIFORNIA. 1935

Excised red clover leaves with their petioles in stoppered vials of water were inoculated and placed, after 4 hours in the laboratory, into preparation dishes containing water, or known concentrations of sulphuric acid. The preparation dishes were placed at known temperature for 24 hours or longer, and the total length of mycelium per mildew colony was determined from measurements of 5 mildew colonies on each of 3 leaflets in each humidity condition tested. According to the results of 4 tests (table 7) with young leaves from the same plant, the size of mildew colonies was less at 0 relative humidity than at 100 per cent relative humidity, but the differences were not great except in 2 tests at 25° C. and 27° C. in which no growth of mycelium occurred at 0 relative humidity. Relative humidities intermediate between 0 and 100 per cent also were tested, but significant differences due even to relatively large differences in relative humidity were observed in only 2 cases.

TABLE 6.—Changes in size of spores during germination, Madison, Wis., 1933

Test fungus	Number of spores measured	Germination interval in hours	Substrate and environment		Average length of spores at beginning of germination interval	Average length of spores at end of germination interval	Average diameter of spores at beginning of germination interval	Average diameter of spores at end of germination interval	Formula used in calculation of spore volume	Increase or decrease in spore volume during germination
<i>Erysiphe polygoni</i> from clover	30	10	17° C.	80% R. H.	34.7	34.1	18.6	15.7	$1/6 \pi d^2$	- 30.0
<i>E. polygoni</i> from cabbage	15	24	20° C.	80% R. H.	44.7	43.7	16.4	13.6	$1/6 \pi d^2$	- 32.8
<i>E. graminis</i> from barley	13	5	20° C.	80% R. H.	33.1	32.3	12.1	11.7	$1/6 \pi d^2$	- 8.9
<i>Uromyces fallens</i> from clover	22	11	19° C.	H ₂ O	23.6	23.6			$1/6 \pi d^3$	0
<i>Colletotrichum trifolii</i>	22	11	19° C.	H ₂ O	14.9	16.4	3.1	3.9	.61 ld^2	+ 74.3
<i>Sclerotinia fructicola</i>	45	6	19° C.	H ₂ O	11.7	13.6	7.5	10.9	$1/6 \pi d^2$	+ 146.0
<i>Ciccnobolus cesatii</i>	25	2	19° C.	H ₂ O	8.5	9.0	2.7	3.3	.61 ld^2	+ 58.0

a $\pi = 3.1414$; l = length of spore; d = diameter of spore.

TABLE 7.—*The effect of relative humidity on the growth of mycelium of clover mildew as determined by four tests conducted at Berkeley, California, 1935*

Date of test	Temperature at time of test	Time interval of test	Total length of hyphae per colony in microns	
			100 per cent relative humidity	0 relative humidity
April 12	22° C.	26 hours	399	210
April 12	22° C.	43 hours	633	451
May 4	27° C.	24 hours	352	0
May 6	16° C.	24 hours	95	108
May 6	25° C.	24 hours	370	0
May 12	16° C.	24 hours	114	99
May 12	25° C.	24 hours	325	248

THE RELATION OF WEATHER TO MILDEW DEVELOPMENT

During the summers of 1930, 1931, and 1932, in Indiana, and 1933, in Wisconsin, the powdery mildew of red clover was prevalent but, apparently, developed most luxuriantly during periods of dry weather. The scarcity of clover mildew in the early part of the season is believed to have been due to the scarcity of inoculum, and not to the weather conditions prevailing, as artificial inoculations in the field in Lafayette, Indiana, in 1932, were successful a month before mildew was found occurring naturally. Clover mildew was always more severe late in the season and on the second crop. During September and October, 1930, 1931, 1932, and 1933, mildew was less severe than during July and August, due, in part it is believed, to the direct injurious effect of the fall rains on mildew development and to the extensive development of *Ciccinobolus cesatii*, a fungus parasite of powdery mildews, which requires free moisture for dissemination of its conidia.

In the region of San Francisco Bay, California, the abundance of powdery mildews and the relative scarcity of other fungous diseases of foliage during the virtually rainless, though somewhat foggy, summer months, is very pronounced. In the dry and relatively fogless summers of the Sacramento and San Joaquin valleys, powdery mildew of grapes is one of the most prevalent and serious (if uncontrolled) diseases. During the rainy season (winter) of the San Francisco Bay region, on the other hand, powdery mildews, with the notable exception of that of cereals, are very scarce, while downy mildews are relatively abundant.

Most clover-mildew inoculations in the field, even those made in the hottest driest hours of sunny days, have been successful; and in no case has failure to secure infection been shown traceable to low humidity,

although several cases of poor infection have resulted. To determine further the relation of weather, especially rain, to mildew development in the field, 2 areas of clover, 3x6 ft. in area, at Madison, Wisconsin, were covered on August 2, 1933, shortly after the first growth of clover had been removed by cutting, with glass sashes at 16 in. above the soil level. These sashes protected the plants from rain, but as no protection was offered on the sides, the humidity and other environmental conditions under the sashes should have been only slightly different from those in the open. The soil below the sashes became rather dry; but, while the protected plants did not make so much growth as did those in the open, they remained in good condition and did not wilt.

There was 4.87 inches of rain with 24 cloudy or partly cloudy days from August 2, when the sashes were put in place, to September 22, when the test was considered completed. On September 22, the mildew on the exposed plants was sparse, or was heavily infected with *Cicinnobolus cesatii*, or both. On the plants protected by the cold frames, the mildew was not parasitized by *Cicinnobolus*, and the vigorous mycelium was producing an abundance of conidia. The mildew development on the protected plants was about twice as severe as on the exposed plants.

Besides the effect of *Cicinnobolus* in controlling the mildew on the exposed plants, the rain, during the period, apparently directly inhibited mildew development. Apart from this test and the one reported later, dealing with conidial dissemination after a rain, the effect of rain on clover mildew in the field has not been tested. With excised clover leaflets on sugar solution, however, mildew was considerably checked or was killed out when inoculated leaflets were atomized with water for a few seconds daily, or only once within 2 days after inoculation. With powdery mildew of peas a single vigorous atomization with water for 15 seconds at 2 or 4 days after inoculation completely washed the mycelium from the leaves and it was not regenerated from the haustoria that remained in the epidermal cells. On mildewed mustard and bean plants exposed to about 2 inches of rain from 3 p. m., February 28, to 8 a. m., March 1, 1935, at Berkeley, California, all the conidiophores were destroyed and much of the mycelium was washed away on the upper surfaces of the leaves, while check plants under a bell jar had a luxuriant development of conidiophores and conidia.

The low soil moisture in the covered area of the above mentioned clover plot probably had little, if any, effect on the mildew development. In duplicated tests in soil cans in the greenhouse, clover mildew developed very slightly better on clover plants grown in soil held at 40 per cent of its water-holding capacity by additions of water to the surface of the soil than

to soil at 20, 60, 80, or 100 per cent of its water-holding capacity. With beans there was no marked difference in the development of powdery mildew on plants growing in soil at 25, 50, 75, and 100 per cent of its water-holding capacity.

Rain may markedly check the normal formation and dissemination of conidia, as would be expected from the injurious action of rain on the conidiophores. A total of 0.32 in. of rain fell from 1:00 p. m. to 2:05 p. m. on July 14, 1933, at Madison, Wisconsin; then there was no rain for several days, and the weather was clear and bright. Exposure of clean glass slides as spore traps in 3 locations in a field of heavily mildewed plants was started at 7 p. m., July 14. The results, based on counts of the conidia on 2.5 sq. cm. on each of the 3 slides, are given in table 8. No conidia were caught until the interval from 11:45 p. m., July 14, to 8 a. m., July 15, when only 2 were caught. The following day (July 15) conidial dis-

TABLE 8.—*Conidial dissemination of Erysiphe polygoni in a clover field after a rain at Madison, Wisconsin, 1933*

Date	Period	Weather	Total number of spores per cm ² per hr.	Average number of spores per cm ² per hr.
July 14	1:08–2:05 p.m.	.32" rain		
	7:00–8:30 p.m.	cool, cloudy	0	0
	8:30–11:45 p.m.	" "	0	0
July 14–15	11:45 p.m.–8:00 a.m.	" "	2	.03
July 15	8:00 a.m.–2:00 p.m.	bright	554	12.1
	2–4:30 p.m.	"	382	20.2
	4:30 p.m.–7 p.m.	"	258	13.8
July 15–16	7:00 p.m.–10:00 a.m.	"	183	1.6
July 16	10:00 a.m.–1:30 p.m.	"	498 ^a	47.4
	1:30 p.m.–3:30 p.m.	"	400 ^b	44.4
	3:30–6:00 p.m.	"	286 ^b	25.4

^a Spores counted only on 1 cm² on each slide.

^b Spores counted only on 1.5 cm² on each slide.

semination was greatly increased but was still only about half that of the third day. Similar results have been secured when mildewed leaves of clover, bean, and mustard were exposed to rain in Berkeley, California, during March, 1935.

High humidity reduces the number of conidia formed. In 15 out of 19 tests on potted plants, or excised leaves with their petioles in water, there was a greater number (300 per cent greater on the average for the 19 tests) of conidia liberated from mildewed clover leaves held at 24 to 49

per cent relative humidity than from leaves held at 94 to 100 per cent relative humidity in laboratory and greenhouse tests at Madison. At Berkeley, similar results were secured with mustard mildew and bean mildew. In the tests at Madison, only conidia that were passively liberated by the mildewed leaves were caught on the spore-trap slides, while in the tests at Berkeley, the test leaves were shaken over spore-trap slides at the bottom of a cylinder. The results, being similar in both types of test, indicate that the cause of the fewer conidia caught from the leaves at high humidity in the Madison tests was not entirely due to their failure to abscise from the conidiophores in the humid atmosphere.

Inoculations performed by dusting conidia onto dry leaves of disease-free plants in the afternoon of clear, sunny days, with no provision for conditions of incubation, have been successful with clover mildew in the field at Lafayette, Indiana, and Madison, Wisconsin, and with sunflower, mustard, peach, and cucumber mildews in the normal outdoor summer environment at Berkeley, California. Similar inoculations were successful in the greenhouse with clover, barley, and cabbage mildews at Madison, and with sunflower, mustard, cucumber, cantaloupe, dandelion, clover, knotweed, bean, barley, oats, and pea mildews at Berkeley. In the laboratory at Berkeley, with relative humidity varying between 25 and 55 per cent, but generally about 44 per cent, mildew infection was successful on excised leaves of rose, sunflower, mustard, cucumber, cantaloupe, clover, and bean. Potted plants of red clover, bean, sunflower, and cantaloupe, inoculated February 16, 1935, with their respective mildews, were placed in chambers with light (about 900 foot candles and a 16-hour day) temperature (25° C.), and 50 per cent relative humidity controlled, and with rapid, forced-air circulation over the plants. All developed a luxuriant growth of mildew, except sunflower, on which it was sparse. In no case has failure to secure infection with any of the powdery mildews studied been traced to low relative humidity.

DISCUSSION

The tolerance of *Erysiphe polygoni* to low humidity as considered in this paper, accords with the diurnal cycle of *Erysiphe polygoni*, previously described. Natural inoculation with *E. polygoni* probably occurs in greatest abundance during the daylight hours of normal sunny days, and infection (the establishment of nutritional relations between host and parasite) probably occurs a few hours later. Free moisture or high humidity are not necessary or desirable at any stage in the conidial life history of this parasite, though very low humidity may be injurious under certain conditions, especially at high temperature. This adaptation to environment should enable this fungus to become a virulent parasite during

the summer season of many climes. That *E. polygoni* is not more abundant than it is may be due to a number of factors, most of which are only imperfectly understood.

Under field conditions, low relative humidity and high temperature are frequently coincident. While *Erysiphe polygoni* is very tolerant to low humidity at low temperature, the injurious action of low relative humidity at high temperature is pronounced. Under laboratory conditions at 100 per cent relative humidity, the maximum temperature for mildew infection of mustard and sunflower was 28° to 31° C., and, for that of clover, pea, and bean, it was between 31° and 34° C., at Berkeley. As temperatures approaching and exceeding these are not uncommon where these crops are grown and these diseases are sometimes prevalent, the caustive fungi can likely tolerate them for short periods (Table 6), but it is probable that at these high temperatures dry weather is injurious to them. Most of the writer's data showing the injurious action of low relative humidity were secured where the conidia were on dry glass slides. In nature, on host leaves, the injury from low humidity would be much less.

Though, under certain conditions, better development of *Erysiphe polygoni* has resulted under conditions of low than under those of high humidity, the writer believes that high humidity is not in itself, markedly injurious to the powdery mildews studied. The luxuriant development of the powdery mildews of clover, pea, bean, rose, dandelion, knotweed, cucumber, cantaloupe, sunflower, and lilac on excised leaves floating on sucrose solution in closed Petri dishes, would preclude this idea. Rather, the writer believes that the forms studied can develop luxuriantly over a wide range of relative humidities and that they are especially well adapted, in contrast with most parasitic fungi, to very dry atmospheric conditions.

The writer does not disagree with the numerous observations by others that certain powdery mildews, including strains of *Erysiphe polygoni*, are frequently more severe in shade or in various types of specially protected environments, but he believes that except where we are concerned with a primary infection center, such as overwintered mycelium, or perithecia, it is generally some factor other than the effect of relative humidity on the fungus that is mainly responsible for this local severity of mildew. The lower temperatures of the air, the greater vigor and succulence of the plants, and the thinner cuticle of the leaves may favor mildew development in some cases. There probably is, also, great variation in different species and strains of the powdery-mildew fungi. The writer's results with *E. polygoni* from mustard, *E. graminis*, from barley, *E. cichoracearum* from sunflower, and *Sphaerotheca pannosa* from rose, indicate that these fungi are considerably less tolerant to low relative humidity than the strains of *E. polygoni* from clover and bean.

SUMMARY

At favorable temperatures, conidia of *Erysiphe polygoni* from red clover and from bean have germinated well at relative humidities ranging from 100 per cent to approximately 0. In some cases more germination resulted at approximately 0 relative humidity or after several hours drying, than at 100 per cent relative humidity, while in others the highest germination occurred at high relative humidity. Even at temperatures favorable for germination, the germinated and nongerminated conidia were soon shrivelled and killed at low relative humidity.

Conidia attached to conidiophores on living mildewed leaves or detached conidia on the surface of healthy leaves were less injured by low relative humidity than conidia on detached mildewed leaves, allowed to dry out, or than detached conidia on dry glass slides. The injurious action of low humidity was increased at high temperatures,

Conidia of *Erysiphe polygoni* and *E. graminis* decreased considerably in volume, but remained turgid at 80 per cent relative humidity. Conidia at 100 per cent relative humidity or floating on water showed a less significant decrease in volume during germination. In contrast conidia of *Colletotrichum trifolii*, *Sclerotinia fructicola*, and *Cicinnobolus cesatii* did not germinate without free water, and increased greatly in volume during germination.

Mycelium of clover mildew grew well at relative humidities ranging from 0 to 100 per cent at low temperatures. At high temperatures and low relative humidity, the mildew colonies were killed in some tests.

Different levels of soil moisture were found to have no definite effect on the development of clover and bean mildew.

Infection with the powdery mildews of clover, bean, cabbage, barley, cantaloupe, delphinium, and mustard has resulted from inoculation made at the lowest relative humidities tested.

Conidiophores of bean and mustard mildew were destroyed during a heavy rain; mildew on peas or beans were injured or destroyed by atomizing the infected leaves with water, and the dissemination of the conidia of clover, bean, and mustard mildew was severely checked by rain. Clover mildew developed better in the field when protected from rain.

General field observations also indicate that *Erysiphe polygoni* and certain other powdery mildews are adversely affected by rain and can develop luxuriantly and possibly better under rather dry conditions.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

IMMUNITY OF VIKING RED CURRANT FROM WHITE PINE BLISTER RUST UNDER FIELD CONDITIONS

GLENN GARDNER HAHN¹

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INTRODUCTION

As an integral part of the investigation concerned with the resistance of Viking (Syn.: Rød Hollandsk Druerips), a Norwegian red-currant variety, to white pine blister rust (*Cronartium ribicola* Fischer), extensive field testing was carried on. These tests in the open, supplemented rigorous artificial experiments in the greenhouse (2, 3),² conducted in 1929 to 1932, in which the introduced variety, cultivated for many years in Norway because of its productiveness, was found to be highly resistant to British and North American strains of blister rust. Moreover, greenhouse results have corroborated field experience with the variety in Norway, where, over a period of many years, Norwegian horticulturists and pathologists have not observed *C. ribicola* fruiting on the leaves of the Viking currant. In Norway, blister rust has been most destructive (4, p. 74-82; 5), and *Pinus strobus* L., extensively planted there, must be considered a dying species.

Taking into account the resistance of Viking to Norwegian strains of blister rust under natural conditions, and its high resistance to British and North American strains under artificial conditions, it was not to be expected that environmental conditions in this country would fundamentally change the physiologic processes of the Norwegian variety to the extent of breaking down resistance to rust. If such an alteration took place, explanation of this phenomenon could be sought in the fact that there were biologic forms of *Cronartium ribicola*, some more pathogenic than others.

To determine this point, Viking was planted widely in the East and West for field testing against strains of blister rust occurring in different parts of the white-pine regions. Optimum conditions for natural infection were obtained in New York, Connecticut, Massachusetts, New Hampshire, Maine, Oregon, and Canada (Ontario). Through the able cooperation of J. F. Martin, E. C. Filler, and other members³ of the Division of Plant

¹ Conducted as a cooperative project between the Bureau of Plant Industry, the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and the Osborn Botanical Laboratory, Yale University.

² Reference is made by number to Literature Cited, p. 875.

³ For their splendid and indispensable cooperation in this experiment, the writer is particularly indebted to the following members of the Division of Plant Disease Control: Connecticut, Mr. J. E. Riley; Maine, Messrs. W. O. Frost, D. S. Curtis, G. H. Kimball, J.

Disease Control, Bureau of Entomology and Plant Quarantine, these field experiments were extended over a period of 3 years, 1932 to 1934. Various planting sites for this experiment were provided, the purpose being to select diverse environmental types in order to study the vegetative development of the Viking and its reaction to disease over a wide range of field conditions in North America.

FIELD EXPERIMENTS WITH WHITE-PINE BLISTER RUST

Plant Material for the Field Test

To test the resistance of Viking under natural conditions, young plants were propagated from cuttings at the pathological greenhouses, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. The cutting stock was obtained in 1930 from Prof. Olav Moen, Norwegian Agricultural High School, Ås, Norway. The young plants were maintained under conditions free of the Japanese beetle at Washington, D. C., and later at the field station of the Division of Plant Exploration and Introduction at Glenn Dale, Maryland, during the winter of 1931-1932. In April, 1932, these 2-year old plants, uniformly thrifty and more or less dormant, were shipped under special permit.⁴ They were planted on selected sites previously prepared for the field experiments.

Planting Sites

Planting sites were selected by members of the Division of Plant Disease Control, due attention being paid to the temporary establishment of Viking in an environment where prevailing winds would be certain to carry abundant aeciospores of white pine blister rust during the spring, to its leaves.⁵

M. White; Massachusetts, Messrs. C. C. Perry, E. M. Brockway, William Clave, G. S. Doore, R. E. Wheeler, K. K. Stimson; New Hampshire, Messrs. L. E. Newman, F. J. Baker, S. H. Boomer, T. L. Kane, T. J. King, L. C. Swain; New York, Messrs. P. E. Barber, B. H. Nichols, H. G. Woodward. The above-mentioned collaborators were responsible for the selection of planting sites, maintenance of the test plots and all the records on the experiment. Mention should be made here of valuable assistance also rendered by Mr. George B. Dorr, Superintendent, Acadia National Park, Mr. C. E. Dow, Dr. A. E. Brower, Entomologist, Maine Forest Service, and Mr. W. I. Leland, Technical Foreman, McFarland C. C. Camp, all of Bar Harbor, Maine; by Dr. H. T. Güssow, Dominion Botanist, Central Experimental Farm, Ottawa, Canada; and by Mr. L. N. Goodding, formerly of the Division of Plant Disease Control, Corvallis, Oregon, now of Soil Conservation Service, and Mr. H. G. Lachmund, formerly of the Division of Forest Pathology.

⁴ Acknowledgment is made of the helpful cooperation of Mr. H. A. Gunning in caring for and preparing the Viking plants for shipment.

⁵ The following cooperators provided planting sites for the Viking field tests: Connecticut, Messrs. E. C. White, Litchfield, W. E. Swift, Cornwall, and D. E. Harris, Salisbury; Massachusetts, Messrs. G. B. Caswell, Holden, E. T. Wilson, East Princeton, Stephen Taylor, Supt., and G. F. Burgess of the New Bedford Water Works, H. L. Bates,

The sites generally were set about with natural pine infection on all sides, the distance from the source of aeciospores varying from 15 to 1500 feet, commonly, 30 to 300 feet.

The following soil types were chosen for planting:

(a) Rich garden soil consisting of a well-drained, sandy loam kept in tillage by the cultivation of other small fruit or vegetable crops (Fig. 1, A).

(b) Heavy clay garden soil lacking fertilization and containing admixtures of peat.

(c) Old pastures or abandoned fields needing fertilization; the soil light and gravelly and competition with weeds and grasses particularly heavy.

(d) Old pastures where the soil was heavy with considerable clay (Fig. 1, B).

(e) Dry, sandy loam in artificial clearings made in dense mixed second growth stands of young white pine and broad-leaf species.

Where fertilization was needed, well-rotted manure was put upon the test plots. Irrigation also was provided wherever possible. The first summer (1932) of the field tests was particularly dry, and drought conditions prevailed quite generally throughout New England. As a consequence, the Viking plants set out in woodland areas suffered from drought injury. Test plants, however, situated on accessible garden sites where water could be applied, were not retarded and made excellent seasonal growth. Garden sites were exposed as rigorously to rust infection as those situated in outlying wooded areas, where, in the main, natural precipitation had to be relied upon.

Inoculum

As has been stated above, the Viking was tested in close proximity to cankered pine, which served as a source of ample aeciospore inoculum in the spring. In one instance (Lewis, N. Y.) some of the currants were planted beneath young diseased white pines, so that fertile infection, if such were possible, could hardly be avoided. In certain instances the test plants were dusted artificially with aeciospores at a time when atmospheric conditions were favorable for infection.

During the field testing, 1932 to 1934, abundant aeciospore inoculum was available. Despite the drought condition of 1932, local infection was evident on *Ribes* in New England, particularly on the wild or pasture goose-

Worthington, H. C. Chapin, Sheffield; Maine, Messrs. G. B. Dorr, Bar Harbor, D. C. Osborn, Camden, G. W. Grover, Freeport, F. Barry and W. D. Whitten, N. Augusta, Mrs. Gertrude Leighton, Bridgton; New Hampshire, Messrs. L. C. Swain, Brentwood, Ernest Hunter, Tuftonboro, Irving Wilson, New Boston, T. B. Southwick, E. Acworth, Paul Dickinson, Lisbon; New York, Messrs. P. E. Barber, Hadley and Corinth, N. H. Harpp, Chestertown, J. E. Crossett, Lewis, Albert Dashnaw, Peru. Acknowledgment is made of this generous cooperation.



FIG. 1. Test plots surrounded by blister-rust-infected pine (*Pinus strobus*), which provided abundant aeciospore inoculum for natural infection of Viking. A. Plot at Chestertown, N. Y. Viking growing on good garden soil. Planted susceptible *Ribes* serving as checks on the presence of rust inoculum, became infected with *Cronartium ribicola*. B. Plot at Sheffield, Mass. Viking growing in an old pasture on heavy soil. Abundant natural infection of blister rust was observed on *Grossularia cynosbati* and cultivated red currants growing in the vicinity. A, photographed July 30; B, Aug. 1, 1934.

berry, *Grossularia cynosbati* (L.) Mill. In Massachusetts⁶ infection was heavier that year than was expected, and in Maine⁷ the American black currant, *Ribes americanum* Mill., showed more than the average amount of infection. That same year black currants, *R. nigrum* L., were also heavily infected in Massachusetts. Abundant aeciospore inoculum likewise was available in 1933 and 1934. The last year of testing, more fruiting cankers were reported in northern New Hampshire⁸ than in any previous year and in New York⁹ blister rust lesions, many of which were large with an abundance of spores, were particularly conspicuous. Urediospore inoculum was also available for infection of the Viking, on the leaves of susceptible *Ribes* either growing naturally about the test plots or planted beside the Viking when the test plants were set out.

Checks on the Field Test

In order to be doubly certain that *Ribes* plants were becoming infected in the immediate neighborhood of the test plants, susceptible "check" plants were planted along with the Viking. In most cases native species such as *Grossularia cynosbati* and *Ribes glandulosum* Grauer (skunk currant) were used; in one instance the very susceptible black currant was utilized. It was the general procedure to remove the infected leaves of the check plants when telia appeared, in order that the rust would not continue to spread to pine in the neighborhood of the plot.

Where susceptible *Ribes* checks were not set out in the experimental plots, statements could be readily substantiated that natural inoculum was available by checking on infection on nearby wild *Ribes*, or on cultivated or escaped red garden currants [*R. sativum* (Reichenb.) Syme (*R. vulgare* Auct. not Lam.)] growing near the test plots. An additional guarantee that ample inoculum was present for infection was provided in certain instances where artificial inoculations in the field were performed. These artificial tests were made despite the fact that it was practically certain that aeciospore inoculum was being distributed, naturally, in the vicinity of the test plants. This additional evidence was sought by the field men to substantiate fully the claim that Viking was immune.

Observations and Records

Records on infection of the Viking (each plant bore a metal tag with an assigned number), together with those for the associated susceptible

⁶ C. C. Perry, in, U. S. Dept. Agr. Plant Indus., Blister Rust News 16: 129, 156, 165. 1932 [Mimeographed].

⁷ J. M. White, in, *Ibid.*, 16: 168. 1932 [Mimeographed].

⁸ T. L. Kane, in, *Ibid.*, 18: 92. 1934 [Mimeographed].

⁹ H. Holcomb, in, *Ibid.*, 18: 95. 1934 [Mimeographed].

check Ribes, were made every fortnight beginning May 15 and ending September 15. Leaves were examined carefully for fertile infection (uredia and telia) and necrotic flecks (2) recorded where fertile infection did not occur.

A record was also kept of the vegetative growth made by the introduced Norwegian variety on the various sites. Measurements were made, May 15 and September 15, of the total length of live stem, living canes and branches per bush, in inches for the year. An average was calculated for the plot, not only for the total length, but, also, for the increase in live stem in inches for the year.

RESULTS OF FIELD TESTS, 1932 TO 1934

In New England and New York

During the period of 3 years' field testing, 927 plants of the introduced Viking were highly resistant to white pine blister rust. They did not produce fertile infection (uredia or telia), although they were exposed to strains of *Cronartium ribicola* in blister-rust areas in New England and New York, where the disease of white pine has been very severe and destructive. As stated above, ample aeciospore inoculum was available during the field experiment, which involved a total of 25 test plots, so that the Viking was thoroughly subjected to natural infection. Susceptible Ribes, set out as check plants, readily became infected. Where Ribes checks were not planted, natural infection, resulting from rust spores distributed over the test plots, could be ascertained by the presence of rust, fruiting on the leaves of wild or cultivated Ribes growing in the vicinity of the Viking test plots. Seven of the total number of test plots were planted near cultivated garden currants, which became naturally infected with the rust, *i.e.*, currants near the test plot located on a rich garden site at Worthington, Massachusetts. Here the Viking grew vigorously, its luxuriant foliage entirely free of uredia or telia, whereas the leaves of red-currant plants growing in close proximity were heavily infected with rust.

As an additional check on the presence of ample inoculum and natural infection, necrotic flecks were observed in many instances on the leaves of the highly resistant Viking. These hypersensitive flecks, as previously described (2), consisted of necrotic leaf cells surrounded by hypertrophied spongy mesophyll and palisade tissue lacking chlorophyll. These flecks were produced only on tender, very young, expanding or recently expanded leaves. They never were observed to produced fertile fruiting. Fully mature leaves of Viking showed no presence, whatsoever, of either necrotic flecks or fertile fruit bodies. In table 1, a summary of results obtained in the field tests is presented.

TABLE 1.—Summary of data on Viking field tests in New England and New York, 1932 to 1934

Viking test plants				Infection data for 3-year period					
Location of plots	Number of bushes		Vegetative growth 1934		Source of aeciospores <i>Cronartium ribicola</i> (distance ft.)	Viking		Susceptible Ribes checks	Infection degree
	Planted 1932	Alive ^b 1934	Av. amount of live stem per bush (inches)	Av. increase in live stem per bush (inches)		Type of Reaction	Necrotic flecks		
<i>Maine</i>									
Bar Harbor									
Plot I	131	129	427.6	283.6	Immune	Positive	{ (8) <i>Ribes glandulosum</i> { (1) <i>R. sativum</i> hybrid American Red Dutch	Abundant	
Plot II	65	65	595.3	302.3	Do	Do	{ (3) <i>R. glandulosum</i> { (1) American Red Dutch	Do	
Camden	20	20	879.6	683.6	Do	Do	<i>R. sativum</i> hybrid	Do	
Freeport	40	40	1004.7	768.7	Do	Do	<i>Grossularia reclinata</i>	Moderate	
North Augusta	25	24	69.5	24.5	Do	Do			
Whitten Plot	15	15	115.9	76.9	Do	Do			
Barry Plot	40	39	149.8	59.8	Do	Do	<i>R. glandulosum</i>	Abundant	
Bridgton							<i>G. cynosbati</i>	Do	
<i>New Hampshire</i>									
New Boston	40	40	298.9	207.9	Do				
Lisbon	40	36	84.9	36.9	Do				
E. Acworth	40	38	75.2	27.2	Do		<i>G. cynosbati</i>	Abundant	
Tuftonboro	40	37	357.7	255.7	Do		Do	Do	
Brentwood	40	40	69.4	32.4	Do				
<i>New York</i>									
Peru	36	32	52.3	9.3	Do	Positive	(4) <i>R. sativum</i> hybrid	Scant	
Lewis	40	38	88.8	11.3	Do	Do	Do	Moderate to abundant	
Chestertown	39	37	208.0	86.0	Do	Do	{ (1) <i>G. reclinata</i> { (1) <i>R. nigrum</i>	Do	
Hadley							{ (1) <i>R. sativum</i> hybrid	Do	
Corinth	40	36	39.4	22.4	Do		{ (1) <i>G. cynosbati</i>	Do	

TABLE 1.—(Continued)

Location of plots	Viking test plants				Source of aeciospores <i>Cronartium ribicola</i> (distance ft.)	Infection data for 3-year period		
	Number of bushes		Vegetative growth 1934			Viking		Susceptible Ribes checks
			Av. amount of live stem per bush (inches)	Av. increase in live stem per bush (inches)		Type of Reac- tion	Necrotic flecks	
	Planted 1932	Alive ^b 1934						
<i>Massachusetts</i>								
Worthington	40	40	199.2	126.2	50	Do	{ <i>R. sativum</i> hybrid <i>G. cynosbati</i> Do <i>R. sativum</i> hybrid <i>R. sativum</i> hybrid <i>R. americanum</i>
Sheffield	40	32	113.1	69.6	30	Do	Positive	
E. Princeton	40	39	47.7	4.7	50	Do	
Holden	40	39	95.3	42.3	1500	Do	
Lakeville	40	40	69.5	14.5	100	Do	
<i>Connecticut</i>								
Cornwall	9	9	290.7	175.2	Vicinity	Do	Positive	(1) <i>R. sativum</i> hybrid
Salisbury	17	17	164.9	98.4	300	Do	Do	
Litchfield	10	10	114.0	46.5	300	Do	Do	
Total: 25 Plots ...	927	892				No production of uredia or telia		Generally moderate to abundant infection white pine blister rust

^a This table is based upon data collected by the cooperating white-pine-blister-rust agents, and compiled by Clave and Stimson under the direction of Filler.

^b Certain of the Viking bushes were destroyed by vandals or killed by drought.

^c Aeciospore inoculum was generally abundant throughout the field test.

^d Numbers in parentheses before Ribes species indicate number of plants actually planted as checks in test plot. Unnumbered Ribes species represent plants already present in the vicinity of the plot when the Viking was set out for testing.

^e Absence of data.

^f Results on the 2 plots at Hadley and Corinth are combined. The checks are those planted in the Hadley plot where 10 Viking plants were set out.

In one instance, the very susceptible *Ribes nigrum* (1), was used as a check plant in the test conducted at Hadley, N. Y. This particular area abounds in cankered white pine, so natural infection of susceptible *Ribes* was to be expected. Such was the case, for in 1932, P. E. Barber stated (written communication) that a black currant, the most congenial host for blister rust, planted beside the Viking, became so heavily infected that it was found necessary to destroy the plant; the Viking leaves, on the other hand, were devoid of rust fructifications. The following year (1933) Barber repeated this experiment, using as an additional check plant, a susceptible red garden currant. He artificially inoculated both the Viking and the checks with aeciospores of white-pine blister rust. The leaves of the *R. nigrum* were reported (written communication) as being as heavily infected as in 1932, and began dropping by the first of August. The red-currant check plant also was infected. The Viking, however, maintained its resistance. This same reaction to rust on the part of the Norwegian variety was again evident in 1934. For the third time the Viking was inoculated with abundant aeciospore inoculum along with the susceptible checks consisting of wild gooseberry, a red currant and a small *R. nigrum* bush. Only the checks became infected.

Similarly, at Lewis, N. Y., in 1933, J. E. Crossett (verbal communication) upon whose farm the Viking had been planted for testing near and beneath cankered young white-pine trees, attempted to infect the Viking artificially by dusting the bushes heavily with aeciospores. He was wholly unsuccessful in obtaining infection on Viking.

The two test plots at Bar Harbor, Maine, (Table 1) were thoroughly exposed to blister rust, the 196 plants of Viking set out there being closely surrounded by diseased pine bearing vigorously fruiting cankers in the spring. The smaller plot was only 15 feet from heavily cankered white pine. In this area, natural infection on the susceptible *Ribes* checks took place readily. The first year of testing, K. K. Stimson artificially inoculated the Viking and one of two American Red Dutch, *Ribes sativum* (*vulgare*) hybrid check plants. These susceptible red-currant plants had been propagated from cutting material received from Norway in 1928, and were fully susceptible to blister rust in artificial inoculation tests (2). Stimson reported (written communication) that both American Red Dutch plants developed heavy infection, although only one of them was artificially inoculated, indicating that natural infection had proceeded as vigorously as artificial infection. Necrotic flecks were found on leaves of the Viking but no telia or uredia.

In figure 2, the Viking currant (Fig. 2, A) is shown in contrast with the American Red Dutch check plant (Fig. 2, B) at Bar Harbor. As stated

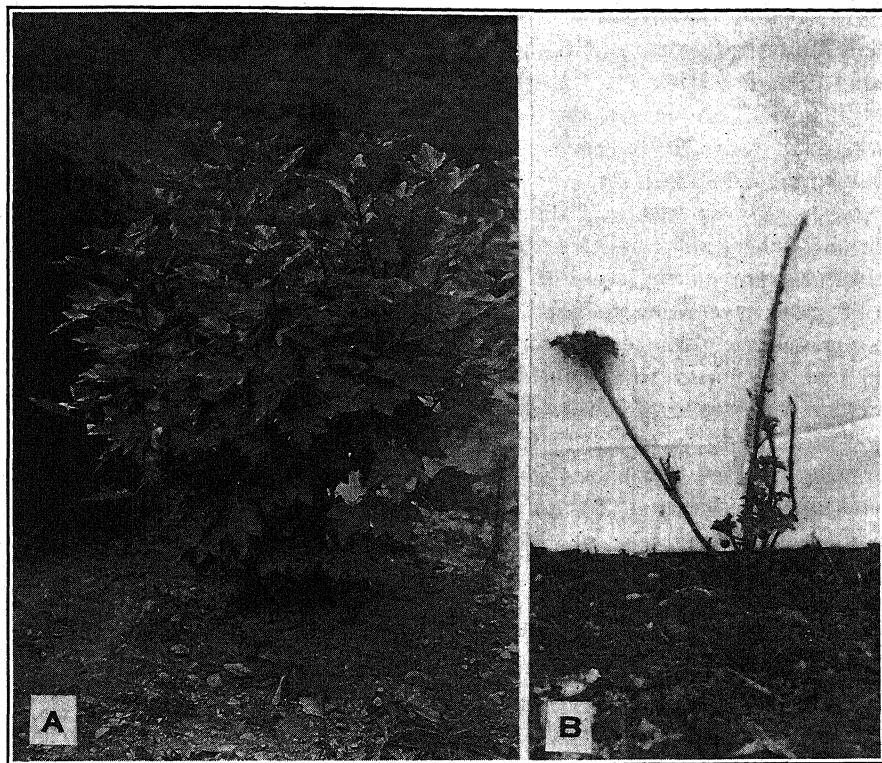


FIG. 2. Highly resistant Viking and susceptible red-currant check growing in Plot II, Bar Harbor, Maine, on the edge of a woodland on heavy clay soil. The plot was surrounded by white pine infected with blister rust. A. Fertile infection of *Cronartium ribicola* was not produced on the luxuriant foliage of Viking. Photographed July 19, 1934. B. Susceptible *Ribes sativum* hybrid var. American Red Dutch, planted as a check beside Viking. This plant became heavily infected each year during the field testing. Continuous stripping (see text) of the leaves bearing telia of *Cronartium ribicola* so reduced the vigor of the plant, that it was mostly dead. The photograph of the plant in flower was made May 21, 1935, on a scale slightly greater than that for A.

above, the leaves of the susceptible check *Ribes*, which became infected with the rust, were removed so that infection to nearby white pine would not continue to spread. Had the Red Dutch plants not been stripped of their infected leaves each year, growth of this susceptible variety would have approximated that of the resistant Viking. Continuous stripping by hand to remove leaves with telia, greatly weakened the check plant so that only a small part of its reduced growth was still alive. It is of course to be admitted, that a growth comparison in figure 2, A and B, is not altogether a fair one. In presenting this figure, it is the author's purpose to convey

to the reader the idea that natural infection of *Ribes* was taking place in this Maine test plot, and that the Viking, subjected to the same rigorous conditions for infection, was rust-free.

Figure 2, A, shows the luxuriant foliage produced at Bar Harbor, where, in 1934, the average increase in live stem per bush was 283.6 and 302.3 inches respectively, in the two test plots (Table 1). This vegetative growth was less than half that produced at Camden and Freeport, Maine, on sites where soil conditions were more favorable. The test plants at Bar Harbor were on the edge of a woodland containing diseased white pine and were, accordingly, partially shaded, so that leaf tissues did not become coarsely thickened, and the dark coppery-green, glossy, rugose type of leaf developed where the Viking was growing in the open on good garden soil, was not formed. Because of the diverse sites chosen for the 25 test plots, all types of leaf texture, that produced in shade, in partial shade, or fully exposed to the sun, were available for field testing.

In a consideration of the average amount of live stem per bush in 1934 and the average increase of vegetative growth for that year (Table 1), it can be readily seen that best growth was obtained in Maine, where the effect of the sea was felt. However, farther south and inland, where the sites were suitable for currants and where good culture prevailed, growth was correspondingly favorable, *i.e.*, Tuftonboro and New Boston, N. H., Worthington, Mass., and Cornwall, Conn. On such sites as those encountered on the Whitten plot, North Augusta, and the one at Bridgton, Maine, or those at Brentwood, N. H., Peru, N. Y., E. Princeton and Lakeville, Mass., where the Viking had been planted in dry, light, gravelly soil with an abundant weed competition, in old pastures or fields, or in woodland clearings, the Norwegian variety showed that it could still keep alive and maintain itself despite the adverse conditions of drought and water shortage encountered during the 3 years of testing. Given proper cultivation on favorable sites, the Viking should grow and fruit successfully over a considerable range of conditions.

In Oregon

Forty plants of the Viking were introduced into Oregon for testing; 10 of them were turned over to G. M. Darrow, Division of Fruit and Vegetable Crops and Diseases, to be planted on the college farm of the State College of Agriculture east of Corvallis, and the remaining 30 taken to a *Ribes* garden established by L. N. Gooding above Rhododendron, Oreg., in the midst of diseased western white pine, *Pinus monticola* Dougl.

In the Rhododendron area, the Viking currant maintained its immunity from western strains of white-pine blister rust. Artificial inoculation experiments with eastern strains of *Cronartium ribicola* taken from *Pinus mon-*

ticola were performed in 1932 in the Rhododendron test plot by J. M. Mielke of the Division of Forest Pathology. While other *Ribes* species and varieties in the garden, which served as check plants, showed heavy infection, the Viking was resistant despite heavy artificial inoculation. In 1934 H. G. Lachmund reported (written communication) that, while all other *Ribes* species had been well infected, no trace of rust fruiting had been found on the Viking and that the plants had become well established and were making good growth. At Corvallis, which is not considered a blister-rust infection area, the Viking was free of rust. There, the variety is being tested as regards its horticultural value for that region.

In Canada

Through the courtesy of H. T. Güssow, Central Experimental Farm, Ottawa, Canada, 6 Viking plants were set out near the currant plantation of the Horticultural Division, containing a large number of red, white, and black currants, together with gooseberries. The Viking was planted within 8 feet of 2 *Ribes nigrum* plants and not far from a block of about 150 bushes of the same species. In 1932 Güssow reported the Viking (written communication) entirely free of rust. The two black-currant bushes were so heavily infected that they were partially defoliated, and the same condition held for the 150 plants, 50 feet away. In 1933, Viking, likewise, did not become infected; and the year later, Güssow stated (written communication) that, while general infection of currants in 1934 had not been severe in the locality, the Viking, although amply exposed to infection, continued to exhibit immunity. This testing with Canadian strains of white-pine blister rust, gave additional evidence that the Viking, whose resistance to Norwegian, British, eastern and western North American rust strains had been demonstrated, was undeniably a highly resistant variety.¹⁰

DISCUSSION

In a recent publication (3, p. 15), in which the immunity of the Viking currant from white-pine blister rust under artificial conditions was reported, it has been pointed out that the utilization of the introduced rust-resistant Viking holds considerable promise, horticulturally, in areas outside of those regions where the growing of white pine is an economic factor. Because of the blister rust, the culture of currants and gooseberries has been restricted or prohibited in these control areas where the northern and western white pines, sugar pine, *Pinus lambertiana* Dougl., and other five-needle (white) pines are important. In those regions, however, where currants and goose-

¹⁰ During the preparation of this paper, field data on the Viking for 1935 have been submitted to the author. There has been no record of any fertile infection on the Viking test plants for that year.

berries are permitted to be grown commercially, or cultivated in private gardens, the introduced Norwegian variety may be an acceptable addition to our horticultural catalog. Moreover, there should be a particular interest in the blister-rust-resistant Viking because of its breeding possibilities.

Currants are natives of cool, moist northern climates. In the United States, as stated by Darrow and Detwiler (1), they succeed best in the northern half of the country and east of the one hundredth meridian. In Norway, where the Viking (see description, 3, p. 3) has been cultivated many years, it still is regarded as the best commercial currant grown, and preferred by the Norwegians to other more recently developed and popularized varieties because it produces an abundant crop of fruit of good quality under their soil and climatic conditions. According to P. Stedje, horticulturist, Norway State Fruit Experiment Station, Hermansverk, Sogn, Norway, the average Viking crop for 1928-1929 (written communication) was 6 kgs. per bush or approximately 6000 kgs. per acre, which, for Norway, is considered a fine yield. Moreover, in Scandinavia, the foliage of the Viking is resistant to most fungus diseases. If, during the horticultural testing of the introduced variety in this country, it is demonstrated that Viking is resistant to the currant borer, *Sesia tipuliformis* Linn., and the imported currant worm, *Pteronidea ribesii* Scopoli., the plant will have added value for those currant regions where these insects are harmful. Fruit worms, particularly the currant maggot, *Epochra canadensis* Loew., (1, p. 2), are very serious pests of currants and gooseberries and have made the production of the fruit difficult in the Rocky Mountain and Pacific Coast States. The currant maggot, for which there is no known means of control, also occurs in some localities in the East, and can be rated along with moisture deficiency and summer heat, as one of the limiting factors in the successful cultivation of currants.

The Viking is now being distributed to horticultural stations in different parts of the country for the purpose of fully testing its horticultural value. Its resistance to heat and drought, as evidenced by the field tests reported in this paper, should make it an acceptable variety for planting in the regions of restricted rainfall, where the number of small fruits that can be grown are exceedingly limited. In the northern Great Plains area and in most parts of the upper Mississippi Valley, where the variety is to be tested, it cannot be expected to produce, however, the abundance or perhaps quality of fruit produced in northern humid climates. In Maine, for instance, where the Viking was tested for resistance to blister rust, the variety made excellent growth, which, with pruning, produced a heavy yield of fruit of good quality. Resistance to disease and insects should give the Viking added advantage for cultivation in dry regions.

The growing¹¹ of the immune Viking currant in white-pine-blister-rust control areas, without present or future danger to protected trees, is, however, open to question, because the seeds of the Viking are germinable and some of the seedlings may not be immune. The planting of large numbers of Viking currants in control areas might, therefore, establish numerous sources of germinable seed from which slightly susceptible plants might become established within these areas in increasing numbers. In view of the vigorous efforts of cooperating Federal and State agencies to control blister rust, by the suppression of all cultivated and wild currants and gooseberries, this might increase the difficulties and cost of maintaining control conditions within these pine-growing areas. It is an open question if the benefits to be obtained from introducing this plant into control areas would offset the increased difficulties and cost of control.

As has been pointed out (2), the origin of the Viking is unknown. The variety, which belongs to the group derived in part from the species *Ribes petraeum* Wulf., may be a hybrid and one of the many forms of the parent stock of the Red Dutch currant (6), cultivated in Europe since the end of the seventeenth century. If it originated by crossing a susceptible with a resistant variety, all the seedlings will not show resistance to rust. The manner of segregation of susceptible and resistant seedlings depends, of course, upon whether or not resistance is a dominant character in the parent. Preliminary inoculation results with open-pollinated Viking seedlings presented below indicate that resistance is dominant. On the other hand, Viking may have originated as a mutation within a variety. In the latter case Viking can be either homozygous or heterozygous for resistance, assuming resistance be dominant. If homozygous, all the seedlings will be resistant; if heterozygous, the seedlings from open-pollinated seeds will probably be segregating, part of them will be resistant and others susceptible.

An investigation is in progress to test the seedlings of the Viking to determine whether they likewise are immune from blister rust, or whether some susceptible seedlings will be produced. This investigation includes the testing of seedlings derived from open-pollinated seed where cross-pollination with nearby escaped susceptible red currants was not precluded, and seedlings derived from selfed-Viking seed. The Viking stock in the New England and New York test plots, have been used as sources for open-pollinated seed. In several instances cross pollination with nearby cultivated red currants in gardens, or escaped from cultivation, was possible.

Preliminary testing has been performed with seedlings derived from Viking seed collected in 1932 by P. Stedje, in the currant collection of the

¹¹ This statement on the growing of an immune currant in blister-rust-control areas was prepared by J. F. Martin, Senior Pathologist, Plant Disease Control, Bureau of Entomology and Plant Quarantine.

Norway State Fruit Experiment Station at Hermansverk. Cross-pollination was not debarred, so that it is likely that foreign pollen from susceptible varieties may have become involved. Among 314 Viking seedlings tested (1933 to 1935), 96 per cent of the open-pollinated seedlings when tested once were immune. Tests were repeated on 184 of these apparently immune seedlings and these were found to be immune on both trials. Additional testing has been performed with 65 of these immune seedlings; 43 were found to be immune on 3 trials, 19 immune on 4 trials, and 3 immune on 5 trials. Additional testing has been performed (1935) with 163 seedlings propagated from open-pollinated Viking seed derived from 12 different sources in New England. This lot of American seedlings showed 97 per cent immune plants. These preliminary experiments demonstrate the fact that we are correct in our assumption that resistance to blister rust is a dominant character in Viking. It will be interesting, indeed, to discover what percentage of resistant plants will appear among selfed-seedlings.

Coming back to the consideration of growing the Viking currant in the white-pine-blister-rust control areas, it is apparent that the problem is one dealing with seedlings of the highly resistant parent. As long as the immune variety is propagated exclusively by cuttings, the resistant characteristic will be perpetuated, but the seedling progeny is another matter. Had we been fortunate in discovering an immune variety, the seed of which would not germinate, there would be very little difficulty in utilizing Viking in the control problem. Tubeuf (7, p. 460) has reported the seed of the plants of the immune Rote Holländische, *Ribes petraeum* hybrid (*R. petraeum* \times *rubrum*), with which he worked, as not germinable. Investigation of Tubeuf's plant might disclose the variety to be horticulturally desirable for cultivation in this country as well as valuable for breeding purposes.

SUMMARY

The resistance (near immunity) of Viking currant (Syn. Rød Hollandsk Druerips), an introduced horticultural variety from Norway, to eastern and western North American strains of white pine blister rust (*Cronartium ribicola*) was demonstrated for 973 plants in a comprehensive field test. This test covered a period of 3 years (1932 to 1934) and consisted of 28 plots distributed throughout blister rust areas in eastern North America—New England, New York and Ontario, Canada—and in Oregon in the Pacific Northwest. In no case were uredia or telia observed. Only necrotic flecks were observed on very young leaves; flecks did not appear on leaves fully mature.

Natural infection was augmented by artificial inoculations in the field, both in the East and in the West. The conditions for natural and artificial inoculation are described.

Results showing a high resistance of Viking to North American and British strains of blister rust corroborate the opinion of Norwegian pathologists and horticulturists who have not observed the Viking to become infected with rust over a period of many years' cultivation in Norway, where blister rust has been severe.

The Viking grew vigorously in northern New England where it made rapid growth on favorable soil sites. Extremely dry conditions were experienced on some of the test plots, but in spite of retardation of growth, the Viking survived, thereby showing its resistance to drought.

Preliminary tests with open-pollinated seedlings of the Viking propagated from Norwegian and American seed showed a very high percentage of immunity from blister rust. These results indicate that rust resistance is a dominant character in the parent.

A discussion of the growing of Viking in blister-rust-control and non-control areas is given.

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THE SUSCEPTIBILITY OF CERTAIN WILD GRASSES TO TILLETIA TRITICI AND TILLETIA LEVIS¹

GEORGE W. FISCHER²

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INTRODUCTION

The incidence of *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn on hosts other than *Triticum* spp. has been reported from time to time in previous years. In 1922, Gaines and Stevenson (9) found F₂ plants of certain rye-wheat and wheat-rye hybrids bearing smutted heads; these proved to be due to *T. tritici*. In 1923 the same authors (10) found *T. tritici* occurring naturally, not only in wheat-rye hybrids, but also in rye, *Secale cereale* L., itself. To determine to what extent rye can serve as a host to *T. tritici*, Gaines and Stevenson (10) artificially inoculated the seed of 9 varieties of winter rye and 14 of spring rye, taking their inoculum from several wheat varieties. The results showed that 4 of the winter ryes and only 1 of the spring ryes were susceptible. The percentage of bunted plants was not high, varying from approximately 2 to 8 per cent. However, similar inoculations of F₂ wheat-rye seed (Turkey × Rosen) yielded approximately 75 per cent bunted plants. Further inoculation experiments indicated that *T. tritici* is weakly parasitic on ordinarily susceptible wheat varieties after passing one generation on rye. It is interesting to note that no infection of rye was secured with inoculum taken from artificially bunted rye. In conclusion these authors (10) suggest that possibly *T. secalis* (Cda.) Kühn and *T. tritici* "are in reality the same fungus growing on different hosts." In 1927, Ducomet (7) also reported the successful inoculation of rye with *T. tritici*, and suggested that *T. secalis* may be only a form of *T. tritici*. Three years later, Lobik (13) recorded the incidence of *T. levis* on rye, but did not consider the spores to be strictly typical of *T. levis*. Bressman, in 1931, (1, 2) succeeded in infecting 2 varieties of rye with "both species of bunt," obtaining 2.2 per cent infection. Of the several physiologic forms employed, Bressman's (4) form IX (*T. tritici*) was decidedly the most virulent.

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² Grateful acknowledgement is made to the following persons: Dr. F. D. Heald for permission to investigate the problem and for helpful advice and criticism accorded; Dr. C. S. Holton for seed, inoculum, and facilities furnished, as well as for helpful advice and suggestions; Mr. Virgil B. Hawk, Agent, Soil Conservation Nurseries, for seed of the many selections of crested and slender wheatgrasses used; Mr. Clarence Seely for obtaining a quantity of the bunted crested wheatgrass for inoculum; and to Dr. A. S. Hitchcock for verifying the identity of some of the grasses used.

Bressman (2) also was of the opinion that *T. secalis* is merely a form of *T. tritici*. In 1932, Dillon Weston (5) further proved the susceptibility of rye to *T. tritici*, and in 1933 Nieves (14) confirmed the reports of the susceptibility of rye to both *T. tritici* and *T. levis*.

Our knowledge of the host range of *Tilletia tritici* and *T. levis* was further extended in 1931-32. The susceptibility of *Aegilops ventricosa* was demonstrated independently by Reichert (16) and by Gaudineau (11). At the same time Bressman (4) successfully infected *Lolium multiflorum* Lam. and *L. perenne* L. with *T. levis*. He used a mixture of 10 physiologic forms of *T. levis* and *T. tritici* for inoculum; however, only the former species appeared in the bunted heads.

Thus it has been shown that *Tilletia levis* and *T. tritici*, long supposed to be specialized to species of *Triticum*, can infect species of *Secale*, *Aegilops* and *Lolium* as well, although the latter hosts are much less susceptible. Up to the present time, no data have been published to further extend our knowledge of the host range of these two important wheat pathogens.

PRESENT INVESTIGATIONS

On July 17, 1934, several bunted heads of crested wheatgrass, *Agropyron cristatum* (L.) Beauv., were received from Mr. H. M. Wanser, formerly Superintendent of the Adams Branch Experiment Station, Lind, Washington. Virtually every floret of each head contained a smut ball, which had the fetid odor often associated with bunt of wheat. Examination of the spores showed them to be morphologically similar to *Tilletia tritici*, and the collection was provisionally assigned to that species.

This incidence of what was apparently wheat bunt on crested wheatgrass incited a number of pertinent questions:

1. Is crested wheatgrass really susceptible to *T. tritici*, as the collection from Lind would indicate?
2. Will a long-lived perennial grass, such as crested wheatgrass, once infected, retain the mycelium of *T. tritici*, producing a crop of bunted heads each year? Bunt, or stinking smut of wheat has heretofore been reported only on cereals and grasses that are either annuals or short-lived perennials.
3. Can the bunt on crested wheatgrass serve as a source of infection of wheat, and if so, to what extent?
4. What other wild or cultivated grasses might also be susceptible to *T. tritici*?
5. Will *T. levis* also find congenial hosts in various of our wild and cultivated grasses?

In order to throw some light on these questions, rather extensive inoculation experiments were begun in the fall of 1934.

WHEAT INOCULATIONS

To test the susceptibility of wheat to the bunt on crested wheatgrass, and at the same time attempt to determine the form identity of the smut collection, the following standard differential varieties were inoculated in the regular manner: Hybrid 128 (C. I. 4512), Turkey (C. I. 6175), Ridit (C. I. 6703), Oro (C. I. 8220), Albit (C. I. 8275), Martin (C. I. 4463), Hohenheimer (C. I. 11458), White Odessa (C. I. 4655), and Hussar (C. I. 4843). The seed was planted in the uniform smut nursery at Pullman.

At the same time, seed of Hard Federation, a spring wheat known to be highly susceptible to most physiologic forms of *Tilletia tritici* and *T. levis* in the Pacific Northwest, was inoculated in flats of soil maintained at 11°–15° C. and the seedlings later transplanted to benches in the greenhouse, where they were grown to maturity.

GRASS INOCULATIONS

The following species of wild and cultivated grasses were tested for susceptibility to *Tilletia levis* and *T. tritici*: *Agropyron cristatum* (L.) Beauv., *A. pauciflorum* (Schwein.) Hitchc. (*A. tenerum* Vasey), *A. repens* (L.) Beauv., *A. spicatum* (Pursh.) Scribn. and Smith, *A. subsecundum* (Link) Hitchc. (*A. caninum* L. Beauv.),³ *Aegilops cylindrica* Host.; *Elymus canadensis* L., *E. condensatus* Presl., *E. glaucus* Buckl.; *Hordeum jubatum* L., *H. murinum* L., *H. nodosum* L.; *Secale montanum* Guss. Most of these are represented by more than one collection. This is especially true of *Agropyron cristatum* and *A. pauciflorum* of which there were twenty-two, and twenty-five collections, respectively. A total of sixty-four wild grass selections were used.

It has long been known that a marked increase in the percentage of infection with other cereal smuts may be obtained by inoculating seed from which the hulls have been removed (commonly spoken of as "dehulled" seed). In the present investigations both normal and dehulled seed of each of the sixty-four selections of wild grasses were inoculated.

The inoculum was of two sorts: (1) *T. tritici*, taken from the original collection of bunted crested wheatgrass from Lind, and (2) a mixture of *T. tritici* and *T. levis*.⁴

³ According to Hitchcock (12) our common bearded wheatgrass has erroneously been referred to *Agropyron caninum*, a distinct European species. His changes in nomenclature are being followed here.

⁴ This mixture was supplied through the courtesy of C. S. Holton and consisted of three distinct forms of *T. tritici*, one of *T. levis* and a virulent hybrid between two smut species, which, according to Dr. Holton, has the pathogenicity of the *tritici* parent, and the morphology of the *levis* parent. These forms were selected because they are very distinct, pathogenically, and therefore, easily separated from a mixture.

Each grass selection was inoculated, therefore, in four ways: (1) Normal seed, with *T. tritici* from crested wheatgrass; (2) dehulled seed, with the same inoculum; (3) normal seed, with a mixture of *T. tritici* and *T. levis*; and (4) dehulled seed, with the same mixture. The inoculations were made by dusting the seeds rather heavily with the inoculum, after which they were planted in boxes and pots of sterilized soil maintained at a temperature of 10–14° C. When the hypocotyls were well above the soil, the containers were removed to the greenhouse. Plants thus inoculated during the winter were later transplanted to pots in the greenhouse. Plants inoculated in the spring were transplanted directly to the field. Since most of the grasses grown in the greenhouse during the winter months failed to head, they likewise were transplanted to the field.

RESULTS

The data obtained from the wheat plantings show that the collection of *Tilletia tritici* from crested wheatgrass is capable of attacking certain wheat varieties, but is not especially virulent. Thus, of the differentials inoculated, only Hybrid 128 was badly smutted. The percentage of infection on this variety was 63.1, on Turkey, 8.53 per cent; the other varieties varied from a trace to 2 per cent. Sixty-five per cent infection was obtained on Hard Federation in the greenhouse. On the basis of the reaction of this collection of *T. tritici* to Albit, Hussar and Ridit, it could be assigned to Bressman's (3) physiologic form VIII, these varieties being essentially immune. The collection likewise conforms to Gaines' (8) physiologic form Tl, on the basis of the reaction to Hard Federation, and to the 9 winter wheat differentials.

The data obtained from the grass plantings described above are not complete, inasmuch as at least 30 per cent of the plants failed to head. This is especially true of the crested wheatgrass selections. Such plants remained in a bushy vegetative condition the entire summer. Culms were sometimes produced, but bore no spikes. However, at least a few plants of nearly every selection headed out so that some information was obtained concerning resistance or susceptibility to bunt. Thus, 15 of the 22 selections of crested wheatgrass proved to be to some degree susceptible to stinking smut of wheat. Twelve of these susceptible selections were susceptible to the collection of *Tilletia tritici* originally taken from crested wheatgrass. Seven selections were susceptible to 1 or more of the 5 forms comprising the mixture of *T. tritici* and *T. levis* described above. Microscopic examination of the bunted heads showed none of the 7 selections to be susceptible to *T. tritici*, whereas 6 were susceptible to *T. levis* or the hybrid form with the *levis* morphology. The form identity in all cases can be determined

only by inoculating back to the 9 differential wheat varieties. Since so many of the plants of crested wheatgrass failed to head, reliable percentages of infection cannot be calculated. For the present it is sufficient to point out the susceptible nature of crested wheatgrass. Percentages of infection, varietal resistance and susceptibility, and physiologic form identity will be determined by investigations now in progress.

The data from the plantings of slender wheatgrass, *Agropyron pauciflorum*, are more reliable, since the majority of these plants headed out with very little of the suppression of flowering observed in crested wheatgrass. Sixteen of the 25 selections of slender wheatgrass proved to be more or less susceptible to bunt. Of these, 11 were susceptible to the original collection of *Tilletia tritici* from crested wheatgrass; 8 were susceptible to 1 or more of the 5 forms of *T. tritici* and *T. levis* in the mixture used for inoculum. Microscopic examination of the spores showed that bunted heads of 6 of the 9 varieties were due to *T. tritici*, and 5 to either *T. levis* or the hybrid form. Three selections were susceptible to both smut species in the mixture. There was an indication of rather wide varietal differences in susceptibility among the selections tested, since 8 selections were bunt-free, while 17 showed 5 to 50 per cent infection. A few selections are apparently quite susceptible, while several are highly resistant, or immune, at least to the physiologic forms of *T. tritici* and *T. levis* used in the present investigation.

In addition to crested wheatgrass and slender wheatgrass, 2 collections of bearded wheatgrass, *Agropyron subsecundum*, were tested and found to be susceptible to *Tilletia levis* and *T. tritici* when artificially inoculated with the mixture of the two smut species.

Of the three species of *Hordeum* tested, only *H. nodosum* proved to be susceptible to bunt. Here again the degree of susceptibility is a matter for conjecture, since only 2 plants survived competition in the greenhouse and matured when transplanted to the field. One of these bore only smutted heads, having been inoculated with the crested wheatgrass collection of *Tilletia tritici*.

The morphology of the bunted heads is usually somewhat different from that of the normal heads. In the case of crested wheatgrass the bunted heads are darker green, shorter, more compact and more plump than the normal ones (Fig. 1, C and D). This effect is due, in part, at least, to the fact that nearly every floret contains a plump, dark green smut ball or "sorus," which spreads the palea and lemma apart in a way that a developing seed would not do. As the heads pass maturity the smut balls shrink and fade somewhat, but, while still green, the bunted heads are usually in marked contrast to the normal ones, thus making recognition

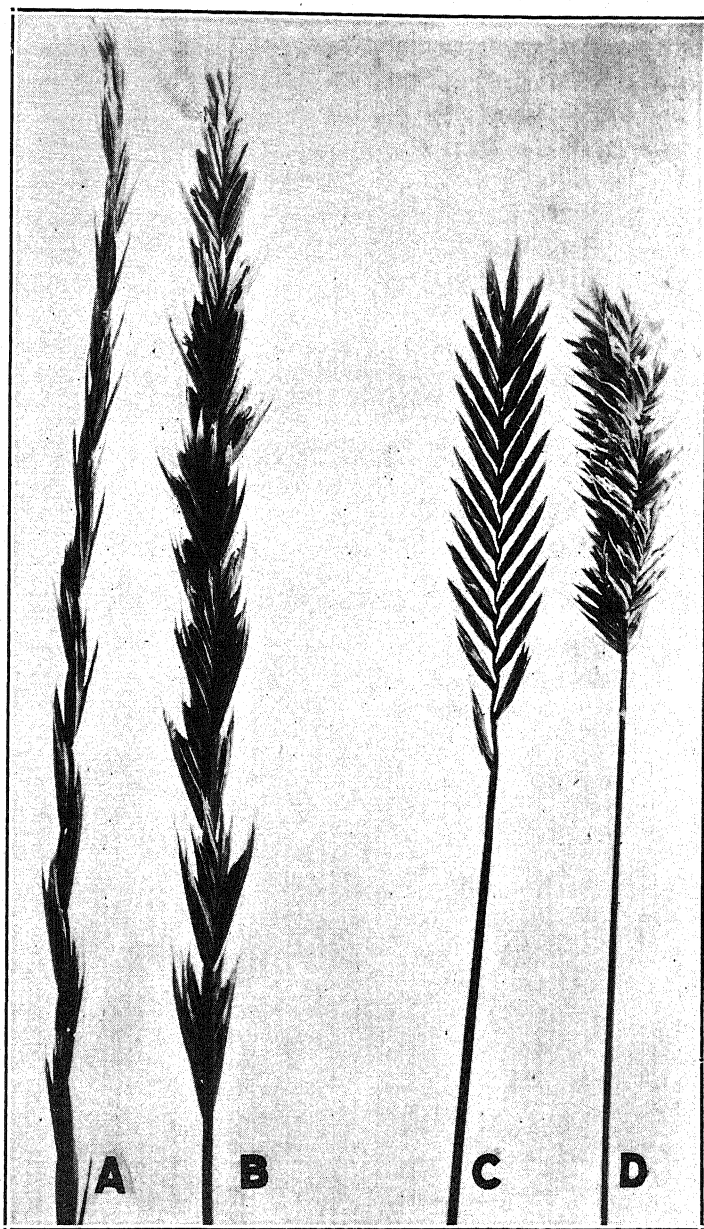


FIG. 1. A and B. *Agropyron pauciflorum*. A. Normal spike. B. Bunted spike. C and D. *A. cristatum*. C. Normal spike. D. Bunted spike.

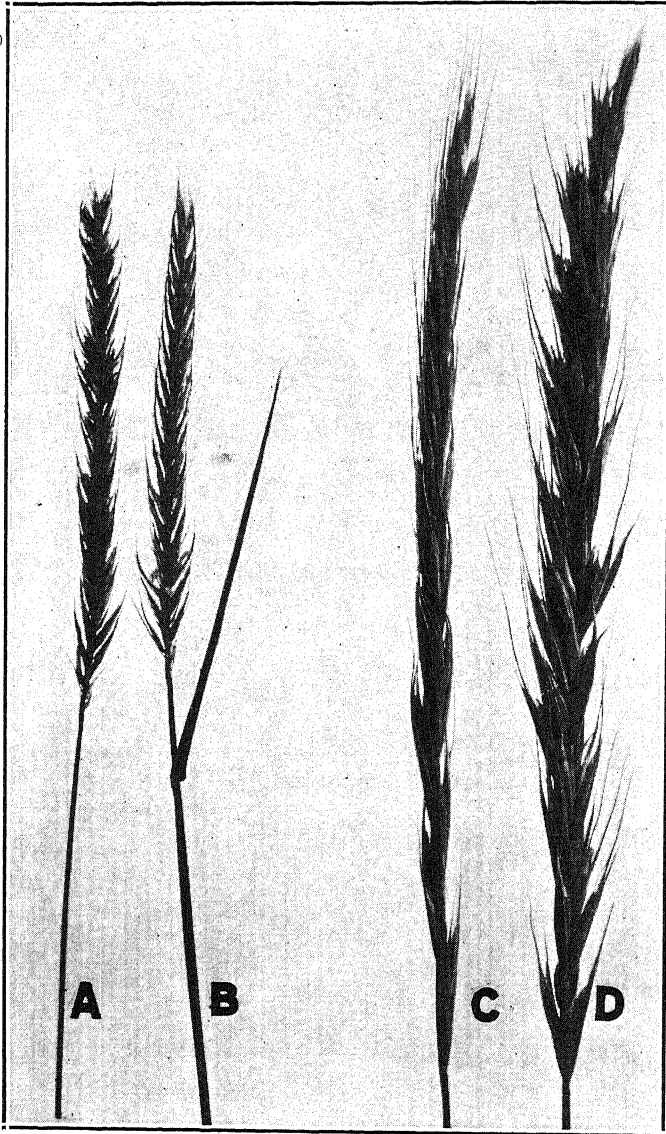


FIG. 2. A and B. *Hordeum nodosum*. A. Bunted spike (due to *Tilletia tritici*). B. Normal spike. C and D. Bearded wheatgrass, *Agropyron subsecundum*. C. Normal spike. D. Bunted spike.

as easy as in the case of bunt of wheat. This is somewhat less true when the bunted heads are due to *Tilletia levis* than to *T. tritici*, since the former generally produces more elongate smut balls, which do not spread the palea and lemma so much. This and other morphological differences between *T. levis* and *T. tritici* have long been observed in bunt of wheat (15).

The recognition of bunted heads of the other susceptible grasses is essentially the same. In the case of slender wheatgrass the bunted heads, in comparison with the normal (Fig. 1, A and B), are more dense and contracted. In some cases, especially when due to *T. levis*, the smut balls are quite elongate, often projecting several millimeters beyond the tips of the palea and lemma. The area exposed to the sun becomes reddish purple, with the result that these smut balls superficially resemble so many ergots. Such bunted heads can easily be recognized from a distance of several feet.

The presence of the long awns on *Agropyron subsecundum* somewhat modifies the appearance of the bunted heads, but such are almost as conspicuous as described for slender wheatgrass. The bunted heads are similarly more dense than the normal ones (Fig. 2, C and D).

On *Hordeum nodosum* the bunted heads are scarcely more conspicuous than normal ones. There is only one smut ball per spikelet, since, even in normal heads, the lateral florets of each spikelet are abortive. Sometimes only about half of the spikelets bear smut balls (Fig. 2, A and B), but in such cases no seeds have been found in the bunt-free spikelets.

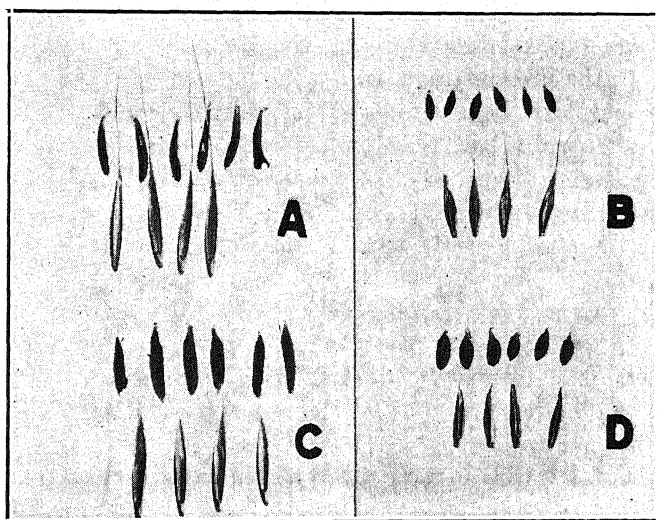


FIG. 3. Smut balls (upper) and normal seeds (lower). A. Bearded wheatgrass. B. Barley grass, *Hordeum nodosum*. C. Slender wheatgrass. D. Crested wheatgrass. Natural size. The slender wheatgrass smut balls contained only *Tilletia levis*; the others contained only *T. tritici*.

Feucht (7) claims that the shape of the smut ball or sorus is influenced more by the normal shape of the seed of any particular wheat variety than by any inherent tendency of *Tilletia levis* to produce elongate sori, or of *T. tritici* to produce short, thick, or rounded sori. Whether this holds true for wheat is perhaps debatable, but it is seen that in the case of the grasses described above, the shape of the smut ball was decidedly influenced by the grass in question. Smut balls on *Agropyron cristatum* tend to be relatively short and thick and rounded, although more so for *T. tritici* than for *T. levis*. Smut balls on *A. pauciflorum* and *A. subsecundum* tend to be decidedly elongate, although sometimes more so for *T. levis*. Figure 3, A-D, illustrates the relative size and shape of smut balls and normal seeds of *A. subsecundum*, *Hordeum nodosum*, *A. pauciflorum* and *A. cristatum*, respectively.

DISCUSSION

It cannot be safely stated yet just how much significance may be attached to the susceptibility of these wild grasses to *Tilletia tritici* and *T. levis*. The criticism may be advanced that the susceptibility demonstrated for certain of these wild grasses was brought about by conditions artificially optimum, and, therefore, such infection may not be a reliable indication of what might be expected under natural conditions. However, the original collection of bunt on crested wheatgrass from Lind, Washington, did not result from artificially inoculated seed, nor subsequently cared for under recognized optimum conditions of soil temperature and moisture. In the present investigation the marked predominance of heads in which every floret bore a well-formed smut ball, and the fact that an infected plant usually bore only bunted heads leads the writer to believe that certain strains of crested wheatgrass and of slender wheatgrass might be quite susceptible under field conditions. Field plantings are in progress in which only normal seed will be inoculated and tested under field conditions.

It would appear, from the data taken during the summer, that hulling the seed does not necessarily promote infection of some grasses. Thus, of 30 bunted plants of crested wheatgrass, 18 resulted from inoculated normal seed, but of 48 bunted plants of slender wheatgrass only 13 resulted from inoculated normal seed.

It remains for future investigations to demonstrate the extent to which the presence of perennially bunted grass plants could be a factor in the control of wheat bunt, as well as a limitation factor in the culture of forage grasses. Field surveys should be made to determine the extent to which bunt occurs naturally on crested and slender wheatgrass.

SUMMARY

Specimens of crested wheatgrass, *Agropyron cristatum*, in which essentially every floret contained a smut ball, have been found in field plantings, and this smut has been shown by its morphology and by inoculations on wheat to be *Tilletia tritici*. Of the 9 winter wheat varieties tested, Hybrid 128 was the most susceptible, showing 63.1 per cent bunted heads. Hard Federation, the only spring wheat tested, yielded 65 per cent bunted heads.

Sixty-four collections of wild grass species of the genera *Agropyron*, *Elymus*, *Hordeum*, *Aegilops*, and *Secale* have been tested for susceptibility to *Tilletia levis* and *T. tritici*. These included 22 selections of crested wheatgrass, and 25 of slender wheatgrass, *Agropyron pauciflorum*.

Crested wheatgrass, slender wheatgrass, and bearded wheatgrass, *Agropyron subsecundum*, were found to be susceptible to certain physiologic forms of both *T. levis* and *T. tritici*. *Hordeum nodosum* was susceptible to *T. tritici*.

Of 22 selections of crested wheatgrass tested, 13 proved to be in some degree susceptible to *T. tritici*, 6 to *T. levis* and 3 to both species.

Fourteen of the 25 selections of slender wheatgrass tested proved to be in some degree susceptible to *T. tritici*, 5 to *T. levis*, and 5 to both species.

There is evidence of considerable varietal resistance and susceptibility among the selections of crested and slender wheatgrasses inoculated with *T. tritici* and *T. levis*.

Species of *Secale*, *Aegilops*, *Lolium*, *Agropyron*, and *Hordeum* have thus far been shown to be to some degree susceptible to bunt of wheat.

DEPARTMENT OF PLANT PATHOLOGY
STATE COLLEGE OF WASHINGTON
PULLMAN, WASHINGTON

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THE STUNT¹ DISEASE OF JAPANESE RICE, THE FIRST PLANT VIROSIS SHOWN TO BE TRANSMITTED BY AN INSECT VECTOR

SABURO KATSURA

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INTRODUCTION

The historic outbreak of the stunt disease of the rice plant in Japan occurred in the 30th year of *Meiji*, 1897, in the leading rice districts of the prefectures Shiga, Kyoto, Hyogo, Okayama, Hiroshima, and Shizuoka. Previously, minor outbreaks had been known locally, since the disease was first discovered in Shiga prefecture in 1883. This disease is transmitted by a leaf hopper known as *Tsumakuro Yakobai*, or *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., and was the first case noted of a biotically transmitted plant virus.

A very early record of severe ravages of leaf hoppers is found in the history of the Japanese famines, which tells of a tragic famine in the 18th year of Kyoho, 1733, when the fertile rice fields of 46 clans of feudal Deimyo were attacked. History records 12,000 people dying of starvation—nearly 5 per cent of the total Japanese population at that time.

When the serious outbreaks of the stunt disease occurred toward the end of the last century, Japan had already begun scientific studies of plant diseases and plant-pest control, but so severe and subtle was the attack of this disease that early science could do little to combat it. Although the people no longer ran the extreme risks of famine, the balance of the nation's food supply was seriously upset.

The menace to the nation's staple food impelled early plant pathologists to initiate strenuous campaigns against the disease that had so suddenly become a major problem.

HISTORICAL REVIEW

The oldest published scientific record concerning the stunt disease appeared in one of the early government gazettes (10) while the disease was attracting little attention. This was written as a formal reply to an interrogation by the Government of the Kyoto prefecture. A paragraph from this report follows—this paragraph being reprinted in an article by Fukushi (8).

"Small insects locally known as 'Unka' (leaf hopper) attacked the field in *Kii-gun, Kyoto*. By their mass attack on the culms and leaves of the rice plant these insects interfere with the normal growth of the plant. Some

¹ Commonly called dwarf disease, but the writer prefers the name stunt disease.

people suspect that the insect causes the stunt disease in this fashion and point to the punctures usually found in the leaves of stunted rice. But it should be remembered that sap sucking insects in general would naturally attack the tender tissue of diseased plants. The insect may add to the damage of the stunt disease, but the cause of the disease should by no means be attributed to its attack."

The author of this comment has never been made known; however it is believed that the report was issued as a sort of warning to growers who, from their observation of the active presence of the leaf hoppers, were prone to conclude that the latter were the responsible factor.

The relation of leaf hoppers to the stunt disease of rice was first experimentally proved in very early days by a local grower, Hatsuho Hashimoto of Shiga prefecture, the center of the disease-stricken area. His little-known work is no small contribution and actually deserves to be considered as one of the very important discoveries. It was because of his failure to publish the results of his studies connecting leaf hoppers with the disease that he is today scientifically unknown, yet his findings more than anything else directed research toward the true solution of the problem.

Hashimoto, according to Ishikawa (9) started his experiments as early as 1883, and the following year discovered the relation between leaf hoppers and the disease. In his investigations he planted rice seedlings in a glass container and put in some leaf hoppers, covering the container with a piece of Victoria lawn to keep the insects within.

The experiments were carried on for two years subsequent to his discovery, so that he could be absolutely sure that his insect was guilty. The experiments were somewhat rudimentary but the basic concept was unique and won a position of paramount importance to the study of this specific virus disease of the rice plant. The attention of other investigators was directed toward various edaphic factors, and at first little notice was taken of Hashimoto's studies.

The Government Gazette (10) printed another misleading paragraph, stressing the importance of soil treatment as a control measure. The production of iron sulphide as the result of oxygen depletion in the soil was postulated to be the chief cause of the checking of normal growth. In order to oxidize the iron sulphide formed in the soil, the report recommended the removal of stagnant water from poorly drained fields, insolation of the soil, and application of unslaked lime.

Much discussion regarding the cause of the disease was entered into by various people, each pointing out different factors, such as poorly-drained fields, drought, low temperature of irrigation water, excess fertilizer, etc.

At this time, so prolific of hypotheses, Hashimoto was quietly bringing his experiments toward a positive result, and finally discovered the impor-

tant clue to the cause of the disease. Perhaps, because of his modesty at not having had pathological training, he made no formal report to any institute and the species of leaf hopper with which he worked was never made known.

In 1883, the government of Shiga prefecture appointed K. Takata to take charge of several experimental plots located in various part of the prefecture. In their first study on transmission of the disease through the soil, Takata and his colleagues definitely ascertained that iron sulphide deposited in the soil bears no relation to the occurrence of the disease.

Takata's attention then was turned to the leaf hoppers, which circumstantial evidence indicated had some possibilities, and that Hashimoto's contemporaneous studies stressed. The first leaf hopper that came to his notice and that he believed to be responsible for the cause of the disease was "Monyokobai," which in later years assumed another name, "Inazuma Yokobai," or *Deltocephalus dorsalis* Motsch. A summary of Takata's work (20) was published in December, 1895 and 1896. This was the first paper in which the etiological significance with respect to the stunt disease was ascribed to an insect pest. In an incredibly short time the main conclusion of the report became known throughout the disease-stricken areas where the spraying of coal oil on the soil became the vogue in checking the newly reported pest.

Takata's papers, although not known to be erroneous in their conclusions, are of historical importance because of the widespread interest they aroused among pathologists. At first his claims found apparent confirmation, but later work by his junior colleagues definitely refuted them. The reported etiological relation between the leaf hopper, *Deltocephalus dorsalis*, and the stunt disease of rice was found to be incorrect.

It is apparent today that the guilty leaf hopper, *Nephotettix apicalis*, although unnoticed, undoubtedly was present in Takata's material, imprisoned in his test chamber; otherwise, no stunt disease could have been produced.

In 1895, the year in which Takata's first report was issued, a branch of the Imperial Agricultural Experiment Station was established in the Shiga prefecture and Takata was appointed its first director. The Shiga station in the first year collected every suspected species of leaf hopper, which belonged to 8 species, including Takata's *Deltocephalus dorsalis*, which was still the center of attention.

In 1898, the station removed 3 species of leaf hopper from the suspected list. The results of this work were published the following year as the first official report of the Shiga-Ken Agricultural Experiment Station (15). The report summarized the general plan of the study of insect pests, concluding:

"The stunt disease of rice is caused by the leaf hoppers, *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., *Deltocephalus dorsalis* Motsch., *Thamnotettix tobæ* Mats., *Cicadula fascifrons* Stal., and *Zygina limbata* Mats., while none of three others, such as *Delphacodes striatellus* Fall., *Tattigonia viridis* L. and *Nisia atrovirens* Leth. was found to be responsible for the occurrence of the disease."

Undoubtedly, the disease was believed at this time to be caused by these named leaf hoppers.

It was, thus, in the 1898 experiments that the really guilty leaf hopper, *Nephotettix apicalis*, was first detected and put on the black list together with 4 others.

In 1900, the station issued Report 2 of the insect pest series, which tells of the narrowing down to *Nephotettix apicalis* as the only leaf hopper capable of producing the true stunt disease of the rice plant. With the isolation of the responsible leaf hopper from the 8 species, the very complex problem seemed to have reached its solution.

The actual causal agent that brings on stunting of rice is as yet unknown. As early as 1900, however, the investigators of the station somewhat hastily concluded that the newly singled out leaf hopper was the etiological factor.

In 1901, N. Takami (19), then the principal plant pathologist in charge of the station, reported his experiments, which he carried on for 6 years, commencing in 1896. In this publication he described the leaf hopper as the sole causal factor, a claim that he later withdrew.

Meanwhile the station (15) continued to extend its insect-pest studies, which, in 1906, reached the sixth report in which the experiments of the previous year worked out under the guidance of Takami were discussed.

This report repeated the hypothesis that Takami outlined in his 1901 paper, and strongly attempted to define the etiology of the disease with his brief that the insect injury was the sole cause of stunt of rice. He based his contention upon his observation that the disease actually was produced by the leaf hopper in his tests carried on for years. This specific leaf hopper, he further stated, was injurious only when it was an inhabitant of a diseased area, while those individuals collected from other places were incapable of producing the disease. In this same year, and shortly after issuing the sixth report, he withdrew his original claims, which his subsequent work had shown to be completely in error.

Takami began to grow suspicious that his leaf hopper acted as a carrier of the disease. It was a bitter state of affairs for him, as his long-held hypothesis had to be altered almost over night. Nevertheless, with such elimination of errors, the studies found their way toward the true solution. Takami is unquestionably one of the men who have outlined important

chapters in the story of the stunt disease of rice. The insect-pest series Nos. 7 and 8, issued in 1908, (15) record many painstaking studies undertaken by the investigators of the Shiga-Ken Agricultural Experiment Station.

The fact about the subtle biological relation regarding the incidence of the disease was first uncovered when the same species of leaf hoppers collected at Tokyo and northern districts outside of the diseased area acquired infectious power when reared on the diseased plants for a period of 5 days, or more. It was in 1906 that the possibility of the leaf hopper acting as an intermediate host was sensed, and, immediately, relevant experiments were started. By 1908, the results yielded substantial confirmation. Meanwhile the Nishi-ga-Hara Imperial Central Agricultural Station situated near the city of Tokyo carried out experiments on the same subject.

As to the stunt disease of rice, the Nishi-ga-Hara station had produced no reliable data up to 1905, when the current hypothesis was that suggested by Takami of the Shiga station. His theory, attributing the cause of the disease to leaf hoppers, was received with much skepticism at Nishi-ga-Hara. The men of the station, under the leadership of H. Ando, decided upon a more thoroughly controlled plan of experimentation. In 1905, the Nishi-ga-Hara station received from Shiga prefecture some stunt-diseased rice plants on which disease-free leaf hoppers, native to Tokyo, were reared in order to produce infected offspring. The offspring, newly hatched, were found to be infectious upon feeding on healthy plants, but another group, whose disease-free parents had fed on healthy plants, showed no sign of the disease. The following year the Nishi-ga-Hara station confirmed the fact that the leaf hopper at Tokyo acquired the infective power if allowed to feed on diseased plants for about 15 days (14).

Another interesting experiment carried out at the Nishi-ga-Hara station concerned the exhaustion of the infective power. The infective leaf hoppers collected at Shiga prefecture were fed on healthy plants that were repeatedly replaced with fresh healthy ones at intervals of from 5 to 7 days. This prevented the leaf hoppers from sucking any diseased juice that might have resulted from infection by their own saliva. As a result of this test it was shown in 1906 that the first progeny was capable of producing the disease, but the second and third were unable to do so. This result, of greatest importance, was announced by the Nishi-ga-Hara station in its report in 1909 (14). The conclusion was then drawn that the stunt disease of the rice plant was of unknown and of mysterious origin, the causative agent of which evidently was carried by the leaf hopper, *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., which performed the rôle of an insect vector. Perhaps the Nishi-ga-Hara central station was the first to prove

the fact, although the Shiga branch station reported the confirmed data in 1908 (15).

Japan's first report telling of a biotically transmitted stunt disease of rice, now known to be a virosis, thus appeared in positive language before the confirmed report of the curly top of beets in the United States. The latter was the first case in literature other than Japanese of a plant virus shown to be transmitted by an insect vector, in this case *Eutettix tenellus* (Baker). The association of *E. tenellus* (Baker) with curly top of sugar beets was first reported by E. D. Ball (2) in 1905, but definite proof of the relation of the insect to the transmission of the disease was first shown by H. B. Shaw (16) in 1910, and later by R. E. Smith and P. A. Bonquet (17, 18) in 1915, and by others.

RECOGNITION AS A VIRUS

The most active discussion then arose regarding the causal agent of stunt. Until comparatively recent years, its type was not understood, but G. Daikuhara in 1902 (3) reported that the insect injury is not the major cause of the disease, and in 1904 (4), he made an unusual prediction regarding the problem. He studied the biochemistry of stunted rice and regarding the nature of the disease stated that it was allied to the sereh disease of sugar cane, to the mosaic disease of tobacco, and very likely to the stigmonose of carnations. In 1905, T. Yonemaru and K. Aso (21) of the Kyoto Agricultural Experiment Station expressed an opinion similar to Daikuhara's, but at that time no reliable data as to the etiology of the disease was available. In 1910, when Andô (1) made his conservative claims in announcing *Nephotettix apicalis* as the carrier of the disease, he stated that the disease was a mysterious one and entirely new to Japanese pathology.

The Nishi-ga-Hara central station's annual reports for 1913-1915 issued in 1917 (14), claim the presence of certain protozoa in the diseased tissues of the plant as well as in the blood of the insect vector. This theory was refuted by M. Kasai (11) of Ohara Institute whose research work in 1924 resulted in finding the Nelson bodies associated with the disease, but no protozoa, he strongly stressed, were to be seen. The bodies, which he called Nelson's type, were later found to be apparently different from those described and figured by Nelson.

The stunt disease of rice became the foremost subject in Japan, and a number of agricultural experiment stations, scattered in various localities, commenced to carry on research work on it. It was assumed that the causal agent of this disease, too small to be microscopically visible, belonged to the virus disease group when considering the symptoms of the disease, occurrence of X bodies, and the mode of transmission.

In 1931, Fukushi (5) reported that certain intracellular bodies were invariably present in the chlorotic tissue of the diseased leaf. His hypothesis is at the present time the most widely accepted one in Japan. Recently his work, mainly carried out in the laboratory at Sapporo University, Hokkaido, Japan, was published (7), describing his improved technique in English for the benefit of western readers.

So far as is known, there has been reported in no other country incidence of the stunt of rice described for Japan. L. O. Kunkel, then at Boyce Thompson Institute, Yonkers, N. Y., was the first investigator in the United States who took an interest in the stunt disease of rice. It was he who in 1926 pointed out the fact that the stunt of Japanese rice apparently belongs to the viroses. He discussed the disease with Fukushi on the occasion of the latter's visit to the United States in 1926. It was shortly after the writer's arrival in Washington, D. C., that he was requested by Kunkel to make an English translation of Takami's paper on the stunt of rice, mentioned in an early paragraph. Kunkel (12) cited the translation in his report, "Aster Yellows," made in 1926, giving recognition to the fact that the stunt disease of Japanese rice was the first known case of a virus disease transmitted by an insect vector.

THE VIRUS WITHIN THE VECTOR

Up to the present, studies have shown that there are approximately 40 species of insects actually capable of transmitting virus diseases from plant to plant. In some cases the insect vectors do not carry the disease just mechanically from plant to plant, but the virus sucked by an insect from a diseased plant seems to be increased by some form of multiplication in the bodies of the carriers. These vectors are mostly cicadellids and aphids; some of them are not yet specifically identified. A comparatively small number of thrips and whitefly vectors also have been reported. Determination of the mode of virus transmission is difficult, such determinations being likely to be largely influenced by individual judgment, since the course of the transmission involves many a complicated bridge. Therefore, we may naturally expect to find some reasonable changes and additions to the category of insect vectors in future investigations, as more data are acquired.

Kunkel (13) expressed his opinion regarding the difficult task of drawing a distinct line between the mechanical and biotic methods of virus transmission from the data that had been obtained. He stated that "Viruses that are not mechanically transmitted, or at least not easily so transmitted, are spread by biological carriers only." According to this, the stunting of rice in Japan is a good example of a biotically transmitted virosis. In 1904, Daikuhara (4) reported the failure to transmit stunt by hypodermic

injections of the diseased sap. Various mechanical means of transmission, including infection with the ground-up bodies of infective leaf hoppers, attempted in 1911 at the Nishi-ga-Hara station, also gave negative results. Fukushi's (7) recent attempts with mechanical transmission in many ways also have failed. As a result of his extended study of stunted rice, he is now impressed strongly with the fact that the attack of this specific virus is possible only when transmitted through the intermediacy of the specific biological carrier.

He reports having carefully sought a clue in various suspected organs in the body of the viruliferous insects. He searched for inclusion bodies, which, in his opinion, might occur within the body of the vector, since he detected the so-called chlamydozos-like bodies in the intercellular bodies found in the tissue of the diseased plants. But, after repeated studies, he failed to find any inclusion bodies, any microorganisms of etiological significance, or any visual evidence of the presence of the virus in the salivary gland and alimentary canal, the egg follicles, the ovarian tubules, the mycetome, or other organs. Nevertheless it is logically conceivable that the virus in some form may be detected in the insect vector. This problem still awaits more extended research.

While it is not known just how the virus is carried in the body of the insect vector, some facts hitherto unknown have been determined by recent studies on the disease carrier. Fukushi's (6, 7) discovery that the virus is carried through the eggs is no doubt of outstanding importance. Such insects are born to the destiny of infecting plants with the virus they carry. He reports that it is not in the egg stage but prior to release of the egg that the progeny receives the virus. The eggs are not infected after they are deposited on the leaf blades. An astonishing phenomenon must have taken place in the body of the viruliferous insect; the attack of the virus in all likelihood occurs during the very early stage of egg formation in the ovary. The assumption that the virus enters the eggs has been sufficiently supported by Fukushi's researches. He believes they are infected in the ovaries and states that "Ovaries, therefore, must not be left out of consideration." This remark may bear much significance and be strikingly near the truth.

Real advancement in scientific research on these delicate biotic carriers is now destined to be made along entirely new lines. The ultramicroscopic entities, either living or non-living, that are now called viruses, present fascinating and important problems that challenge the intelligence and shrewdness of workers in the fields of phytopathology and entomology.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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COMPARISON OF DERIVATIVES FROM DISTINCTIVE STRAINS OF TOBACCO-MOSAIC VIRUS¹

FRANCIS O. HOLMES

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It recently has become evident that there exist closely related strains of many viruses. The strains of any one virus possess many properties in common, generally having, for example, essentially the same host ranges, similar thermal inactivation temperatures, and common antigenic properties. They usually differ from one another only slightly, as in symptom expression on host plants. Strains of one virus differ as a group from strains of another virus in respect to most of their characteristics. An essential feature of virus strains is that they may be transmitted serially without loss of distinctive characteristics. It is generally assumed, and in some cases known, that they have been derived from preexisting parent strains (3, 4, 5, 6). The extent to which strains transmit their distinctive characteristics to variants arising from them is not known. Experiments were undertaken for the purpose of determining this for tobacco-mosaic virus (*tobacco virus 1*) in the case of 2 previously described strains, the *masked*, and the *distorting* from which the *masked* originated.² The experiments were designed to determine whether or not yellow-type strains derived from the *masked* strain of tobacco-mosaic virus would be closely comparable with those derived from the *distorting*, without this previous transformation into the *masked* strain.

STRAINS OF TOBACCO-MOSAIC VIRUS USED AS SOURCES OF DERIVATIVES

Masked tobacco-mosaic virus differs from *distorting*, an ordinary field-type strain from which it was derived, by such characteristics as failure to induce mottling and distortion in infected plants of *Nicotiana tabacum* L. and a number of other species that are typically mottled and distorted by field-type virus. It also differs from *distorting* virus by a tendency to invade young leaves less extensively in *N. tabacum*, as well as to remain

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² For the sake of conciseness, the italicized words "*distorting*" and "*masked*" will be used as names of the *distorting* and *masked* strains, respectively. This will aid in brevity and help to avoid the false implication that *masked* virus is characterized by inability to produce symptoms in all hosts mentioned. The name was chosen originally because this strain produces only *masked* symptoms in *Nicotiana tabacum*; in certain other species, as in *N. glutinosa*, it produces conspicuous symptoms.

localized in the inoculated leaf in young plants of *N. rustica* L. It possesses the basic characteristics of tobacco-mosaic virus; high thermal inactivation point, resistance to inactivation when stored dry or in frozen juice, and ability to infect at high dilution (3, pp. 855-858). Furthermore, Chester (1, 2) has shown that it reacts with the sera of animals previously injected with distorting-type tobacco-mosaic virus, giving a characteristic neutralization reaction (1, p. 1200) as well as complement fixation and precipitin reactions (2, p. 694). Occasional yellow spots appearing on otherwise symptomless leaves infected with *masked* virus are potential sources of yellow-type strains (3, pp. 845-846). Sometimes these yellow spots, like those appearing on plants affected by ordinary tobacco mosaic, become semi-necrotic or fully necrotic in type. They will be referred to here merely as yellow spots, since they are usually yellow when first seen. Since the time of its derivation from *distorting* virus, the *masked* strain has been transferred frequently and preserved at times in frozen juice samples or dried leaves. As an indication of the probable degree of purity of the stock in hand at the beginning of the present experiments, an outline will be given of recent transfers. Virus was transferred to plants of *N. glutinosa* L. and isolated again from a single necrotic primary lesion. It was then transferred to *N. tabacum* and carried through 12 serial passages in that species, its presence in these symptomless plants being confirmed from time to time by inoculation of *N. glutinosa* plants. Subsequently it was transferred to plants of *N. langsdorffii* Weinm., and carried through 5 serial passages in single necrotic lesions. Then it was transferred again to plants of *N. tabacum* and preserved in dried leaves. Since even one transfer from a necrotic lesion in *N. langsdorffii* or *N. glutinosa* suffices to separate intentionally mixed strains, it is believed that the final stock of *masked*-strain virus represented essentially a pure line. It is understood that very small amounts of new derivative strains may have appeared in any transfer, and may be represented in the final sample, but there has been no indication of their presence in this one, and no reason to believe that they need be taken into account.

The *distorting* strain (3, p. 847) has the properties of typical tobacco-mosaic virus; these are too well known to be recounted here. The name *distorting* was applied to this particular isolated strain to distinguish it from other isolated strains, also of distorting type, and possessing the generally recognized properties of typical tobacco-mosaic virus, but differing from the strain in question by producing more stunting in *Nicotiana tabacum*, flecks of necrosis in invaded leaves, less distortion of leaves, or other distinctive symptoms. The stock of *distorting*-strain virus used in these experiments was one that had been obtained after repeated isolation

from single lesions in plants of *N. glutinosa* infected with highly diluted samples of juice of mosaic plants.

DERIVATION OF YELLOW-MOSAIC STRAINS

New yellow-type strains were derived from the *masked* and *distorting* strains as follows. Sets of young plants of *Nicotiana tabacum* var. Turkish were inoculated with the previously purified stocks of tobacco-mosaic virus of the *masked* strain and the *distorting* strain respectively, from tobacco plants that had not been diseased long enough to show yellow spots. Healthy control plants were retained in numbers equal to those in infected

TABLE 1.—*Summary of experiments on derivation of yellow-type strains from two known strains of tobacco-mosaic virus*

Experiment number	Strains of tobacco-mosaic virus, used as sources of yellow type derivatives	Yellow-type strains isolated			Failures to obtain yellow-type strains	Totals of attempts to isolate new strains ^b
		Systemic, fully invasive	Systemic, not fully invasive	Localized only ^a		
1	Distorting strain	23	5	3	13	44
2	Masked strain	0	4	4	17	25
3	“ “	0	7	3	31	41
4	“ “	0	2	2	36	40

^a In addition to those recorded in this column, 7 nonsystemic yellow-type strains were obtained in exp. 1, 4 in exp. 3, and 1 in exp. 4, in sets of transfers that also yielded systemic yellow-mosaic strains.

^b For each attempt to derive a new strain of virus, 24 Turkish tobacco plants were inoculated, by two pin punctures in each, from a yellow or necrotic spot on a plant infected with the indicated one of the two strains of tobacco-mosaic virus used as sources.

sets; no mottling symptoms, and no yellow spots appeared on these uninfectd plants. As soon as yellow spots appeared on the inoculated plants, isolation of new strains was attempted. Sometimes more than one yellow spot appeared on a single infected plant, but only one was used in each case. From each tested yellow spot, transfers were made by a method essentially like that of Jensen (4, p. 965) using a sterilized No. 00 insect pin. One leaf of each of 24 healthy young tobacco plants received two inoculation punctures near its base.

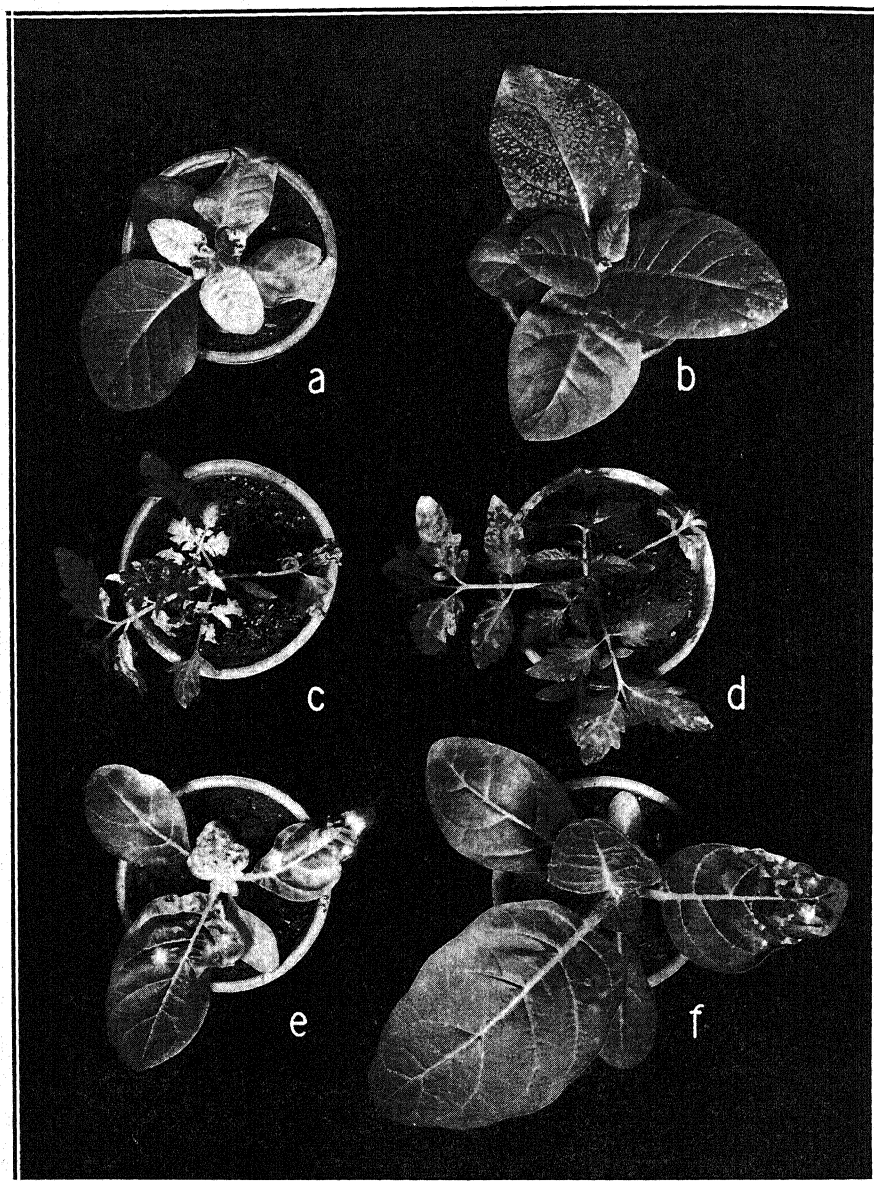
In table 1 are shown the summarized results of several experiments. Yellow-type strains were isolated from 31 separate tobacco plants previously infected with *distorting*-strain virus. These yellow-mosaic strains showed considerable variation in invasiveness, that is, in ability to invade

and affect the younger leaves of infected plants, as well as in degree of yellowness of affected leaves, tendency to produce necrosis, and other characteristics. The variation was similar to that recognized previously from reports of Jensen (4, 5) and McKinney (6) for yellow-mosaic strains derived from field-type tobacco-mosaic virus. Of the 31 yellow-type strains, 23 showed a sufficient degree of invasiveness to cause puckering of young leaves and distortion of tips of leaves at the top of the plant at the time of onset of mottling symptoms. These are described in the table as fully invasive, systemic yellow-type strains. Five additional strains produced systemic infections, but did not cause puckering and distortion of young leaves. They are represented as a separate class in the table, as are also 3 that did not produce any systemic symptoms. Yellow-type strains were isolated from 22 separate Turkish tobacco plants previously infected with *masked*-strain virus. Of these, 13 strains proved capable of systemic spread in tobacco, but none showed a high degree of invasiveness. The young leaves of plants infected with yellow-type strains derived from the *masked* virus were either wholly green, or green except for small yellow areas near their tips. Older leaves, although sometimes very yellow, did not lose their natural flatness and were not distorted at their tips. Typical symptoms produced by systemic yellow-mosaic strains derived from *distorting* and *masked* strains are represented in figure 1.

The first two experiments recorded in table 1 were performed simultaneously. In these experiments, 50 tobacco plants were inoculated with the *masked* and a like number with the *distorting* virus. It will be seen from the numbers of attempts to isolate new strains, listed in the table, that more plants infected with the *distorting*-strain (44 altogether) than with the *masked* strain (25 altogether) developed one or more yellow spots during the course of the experiment. About the same number of localized yellow strains, and of systemic, but relatively uninvasive, yellow strains were isolated from each of these sets. The main difference between the sets was the complete failure to obtain fully invasive yellow-mosaic strains from the *masked* strain.

The third and fourth experiments in table 1 were done under conditions of low susceptibility to inoculation. They were performed for the purpose of increasing the total number of isolations of systemic yellow-mosaic strains from the *masked* virus, and were not accompanied by attempts to isolate from *distorting* virus. More failures to secure yellow-type strains occurred, but otherwise the results were comparable with those previously secured, no fully invasive yellow-mosaic strains being obtained from yellow spots on plants infected with *masked* virus.

Other experiments have served to confirm this result. In general, fully invasive yellow-mosaic strains have been derived repeatedly from *dis-*



Photographed by J. A. Carlile

FIG. 1. Symptoms produced by typical yellow-mosaic strains derived respectively from *distorting* strain (a, c, e) and *masked* strain (b, d, f) of tobacco-mosaic virus, in *Nicotiana tabacum* (a, b), *Lycopersicon esculentum* Mill. (c, d), and *N. rustica* (e, f).

torting virus; but among yellow-mosaic strains derived from *masked* virus there have been none that appeared to be fully invasive.

It has been found that derived strains, if mixed with each other or with original stocks, can be separated by transfer from necrotic primary lesions on such plants as *Nicotiana glutinosa* and *N. langsdorffii*. As a routine technique newly derived strains were in most cases transferred to *N. langsdorffii*, and reisolated from single necrotic primary lesions.

Transfer of strains from necrotic lesions of *Nicotiana langsdorffii* to plants of *N. tabacum* was accomplished by modification of the cork-borer technique previously described by Jensen (4, p. 965). The modification consisted essentially in substituting for the cork borers sharpened tubes made from $\frac{1}{4}$ -inch brass tubing, and in their use not only to cut out single necrotic lesions and surrounding tissues, but to crush the tissues, and then to inoculate leaves of healthy plants of *N. tabacum*. During the processes of cutting out lesions and later inoculation, microscope slides of glass were used to support the leaves. Preceding inoculation, the excised leaf material was crushed in a drop of water on the glass slide, by rolling and rubbing the brass tube over it. After use, both instruments were rinsed and sterilized by boiling. The technique is rapid and continuous, allowing transfer from many necrotic lesions without recourse to autoclaving of more bulky instruments, and with no danger of contamination of plants, which are touched only by instruments designated for the particular transfer.

After such transfer to *Nicotiana tabacum* the degrees of yellowing and of invasiveness were again observed in this host species, for each new strain of virus. Virus samples in leaves of *N. tabacum* were preserved in 4-inch lengths of cellophane tubing, with ends closed by twisting. The leaves lost moisture rapidly; when thoroughly air-dried, the closed tube and included leaf fragments were filed in seed envelopes. The imperviousness of cellophane tubing to virus ensured against contamination.

In general the invasiveness of the yellow-mosaic strains derived from *masked*-strain virus did not change during the processes connected with reisolation or storage. The only observed change toward greater invasiveness occurred while a strain recently derived from the *masked* strain was in a tobacco plant. It increased in invasiveness, as shown by subsequent transfer, but comparison with strains derived from both original stocks showed that it could not be classed as fully invasive. It did not cause puckering or yellowing symptoms on young leaves of tobacco plants. Although further variation of strains derived from the *masked* strain might in time disclose full recovery of invasiveness, such recovery has not yet been observed.

Attention was primarily directed to systemic yellow-type strains in these experiments. Whether or not the nonsystemic, *i.e.*, localized, yellow-type strains derived from one source differed as a class from those derived from

the other has not been investigated. A few words with regard to the localized strains may, however, be justifiable. When both localized and systemic yellow strains were isolated from a single yellow spot, only the more invasive form was recorded in the table. Additional nonsystemic yellow-type strains were obtained in some sets of transfers from single yellow spots that also yielded systemic yellow-mosaic strains; these are indicated in the first footnote below the table. All yellow-type strains of tobacco-mosaic virus that were characterized by little or no movement except by slow extension of the primary lesion proved difficult to transfer by the usual rubbing methods. Their transfer was greatly facilitated, however, by sprinkling leaves before inoculation with No. 320 carborundum powder. This was done in accordance with the recommendation of Rawlins and Tompkins (7), who used carborundum powder of a similar size (No. 370) for transfer of tomato spotted-wilt and other viruses.

The usefulness of *Nicotiana rustica* in distinguishing between tobacco-mosaic strains having varied abilities to spread into young tissues of host plants has been pointed out previously (3, p. 849). Since evidence indicated that strains derived from the *masked* strain differed in regard to invasiveness from those derived from the *distorting* strain, *N. rustica* was used to supply additional visual evidence of this difference. Young plants, still in the rosette stage of their development, were inoculated by rubbing. Strains of virus capable of extensive systemic movement in *N. tabacum* caused death of these *N. rustica* plants as a result of numerous necrotic secondary lesions in young leaves and stems. Strains of virus of more limited ability to move systemically caused little or no stunting, and gave few or no secondary necrotic lesions. Photographs showing the effects produced on *N. rustica* by yellow-mosaic strains derived from *distorting* and *masked* strains are represented in figure 1 (e, f).

DISCUSSION

The *masked* strain of tobacco-mosaic virus, used in these experiments as one source of yellow-mosaic strains, was originally derived from the *distorting* strain of the same virus. Although it shares certain fundamental attributes with the *distorting* strain, it has been shown to differ from this strain in several respects (3). It does not produce yellow patterns in affected leaves. It does not spread into and affect young tissues in plants of *Nicotiana tabacum*. It multiplies at temperatures too high to permit increase of the *distorting* strain.

The inability of the *masked* strain to spread freely in young tissues of such plants as *Nicotiana tabacum* appears to be due to loss of a quality, here called invasiveness, which was inherent in the original *distorting*-strain virus. The characteristic of invasiveness, once lost in the change from the *distort-*

ing to the *masked* strain, failed to reappear among derivatives of yellow-mosaic type obtained from the *masked* strain, although it was represented in a majority of yellow-mosaic strains derived directly from the original *distorting*-strain virus. The lack of invasiveness, characteristic of the *masked* strain, was inherited, so to speak, by yellow-mosaic derivatives obtained from it. Thus yellowing and invasiveness may be thought of as independent units, however complex each may prove to be upon further analysis. The question arises whether these and other possible unit differences between strains of tobacco-mosaic virus may not be comparable either to genic differences between parts of chromosomes within plants and animals, or to differences between single allelomorphic genes.

Other differences between the *masked* strain and the *distorting* strain, such as the difference between maximum temperatures for virus increase, and the striking difference in tendency to yellow chlorophyll in invaded tissues of *Nicotiana tabacum*, have not yet been demonstrated to act independently, and may in some instances be due to combined effects of several or many structural changes. Future work may show some of them to be passed on without change to derived strains, and others may fail to be represented in any simple way in derivatives.

The work here reported has demonstrated that from a symptomless strain of virus there may be obtained numerous derivatives capable of causing severe symptoms, at least with regard to interference with the chlorophyll mechanism of the host plant. This change from inability to affect chlorophyll to ability to injure it conspicuously is perhaps only of technical interest in the case of tobacco-mosaic virus, severe-symptom strains of which are already world-wide in distribution; but the principles involved may be applicable to consideration of other viruses, in which similar changes may be of practical importance. Such procedures as temporary growth of introduced plants in quarantine, coupled with selection by visual inspection, may be insufficient to prevent importation of vegetatively propagated plants carrying symptomless strains of viruses. After introduction, continued culture may give opportunity for destructive variant strains to arise, and to spread from plant to plant within a country not previously invaded.

Several means may be suggested as possible aids to detection of symptomless strains of viruses. One means of detection depends on the fact that certain plant species serve as dependable indicators of some viruses, by displaying conspicuous and characteristic symptoms irrespective of the strain of the virus used to infect them. A second means of detection involves the use of serum reactions (1, 2). A third means of detection is by observation of occasional occurrence of small irregular-shape yellow spots, similar to those used in the present work as sources of yellow-mosaic variant strains of tobacco-mosaic virus. Isolated yellow spots, in the absence of all other

symptoms, are not usually considered characteristic symptoms of virus diseases, yet appearance of such yellow spots, representing formation of variants, may disclose the presence of virus strains, which of themselves do not visibly affect invaded tissues.

SUMMARY

Yellow-mosaic strains derived from the *masked* strain of tobacco-mosaic virus, although not confined to primary lesions, were found characteristically to lack the high degree of invasiveness possessed by many yellow-mosaic strains derived directly from the *distorting* strain of tobacco-mosaic virus. The uninvasive character that was obtained at the time of isolation of the *masked* strain was thus retained in the systemic yellow-type derivatives of this strain.

Changes to yellow-mosaic type and to invasive type appear to be independent and may represent unit differences in the structure of the virus similar to unit differences in genetic structures of plants and animals.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

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THE CALORIFIC VALUE OF DECAYED CORDWOOD

M. T. HILBORN¹

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INTRODUCTION

It is well known that fungous decay of wood is a complicated process. Such decay and its rate vary with the kind of fungus, the species and the part of the host, the environmental conditions, and the stage of decay that has been reached by the process. It is also well known that the various changes occurring during the stages of decay include a reduction in the weight of the wood; changes in the chemical composition of the wood; an increase in the water-absorbing capacity of the wood; and an ultimate reduction in the volume of the wood.

A method of measuring fungous decay in wood should be one that measures the progress of these changes. Much of the previous work has been concerned primarily with the changes in specific-gravity values, with changes in chemical composition, or with changes in breaking strength. Since calorific value depends upon the chemical nature of the cell wall and cell contents, as well as upon the amount of these substances present, its determination appears to be applicable in evaluating the fungous decay of wood or other plant materials. Since it measures the amount of wood residue remaining rather than the amount lost it also fulfills the suggestion made in the recent article by Buckman (2).

Lehmann and Scheible (5) enumerate 3 methods of investigating the rate of decay in wood: (1) production of CO_2 as an index of the destruction of wood substance, (2) change in specific gravity, and (3) loss in calorific value. All of these methods were employed by them in a controlled laboratory experiment where determinations could be made on inoculated material and on checks. It may be assumed that results obtained in a controlled laboratory experiment are not always applicable to conditions as they exist in the forest or in storage sheds for forest products.

It is the purpose of this paper to apply the method of determining loss in calorific value to a study of decay in 3 species of hardwoods stored in the open in Maine for 4 years under different conditions. The changes measured were the specific gravity when air dried, the calorific value, and the moisture content. On the basis of these measured changes and several

¹ The writer is indebted to Dr. F. H. Steinmetz, University of Maine, for advice and other assistance during the course of the investigation and for assistance in the preparation of the manuscript; to Dr. D. Folsom, Maine Agricultural Experiment Station for aid in the preparation of the manuscript; and to Mr. R. M. Lindgren, Division of Forest Pathology, Bureau of Plant Industry, U. S. Dept. of Agr. for aid in obtaining literature.

reasonable assumptions, calculations were made of the practical fuel value of the wood as influenced by species of wood, splitting, part of the tree, type of storage, and length of storage period.

METHODS AND PROCEDURE

A cord of wood consisting of split and unsplit pieces of three hardwood species, namely: red maple, *Acer rubrum* L.; paper birch, *Betula papyrifera* Marsh.; and beech, *Fagus grandifolia* Ehrh., was purchased in June 1930. This wood had been cut in 4-ft. lengths the preceding winter, and it is assumed that due to the low temperature no measurable amount of decay had taken place during the intervening time.

One half of this cord, consisting of approximately equal numbers of split and unsplit pieces, was piled in the woods at Pushaw Pond, Maine, while the other half was piled in an open field at Orono, Maine, approximately 10 miles away. Throughout this paper these two locations will be referred to as "woods" and "open field" respectively. The piling was done according to approved forestry practice, bark side up on the split pieces. Semi-annually a representative stick (4 feet long) of split and round pieces of each of the species was brought into the laboratory from each location and cut into 6-inch lengths, one of which was chosen as a representative sample for the 4-foot stick and used for all determinations. Laboratory air-dry weights were determined. The specific gravity of the air-dry samples was determined by displacement, after removing the bark from the 6-inch piece. The sample for the calorific value was obtained by drilling a series of holes 1-inch apart with a half-inch auger for the entire length of the 6-inch piece. This was drilled at right angles to the plane of cleavage, and as a result the dry, split edge on the split piece was not used in these samples. The drillings were carefully collected and ground into a fine powder. The moisture content of this sample was determined by drying to constant weight at 105° C. The ash was determined by incinerating a 2-gram sample in a muffle furnace. Aliquot parts, approximately 1 gram each, were taken of this oven-dried powder. These were compacted into pellets and the calorific values were determined in an Emerson Bomb Calorimeter. The data used for further calculations were the averages of two good checks, *i.e.*, within 10 calories variance.

DATA AND DISCUSSION

The values obtained for specific gravity are shown in figure 1. The writer has no proved explanation to offer for the differences shown between the split and unsplit pieces. When second-growth hardwoods are cut for cordwood in Maine it is the common practice to split the trunk wood and leave the smaller branch wood as round pieces. Paul (6) has found in test-

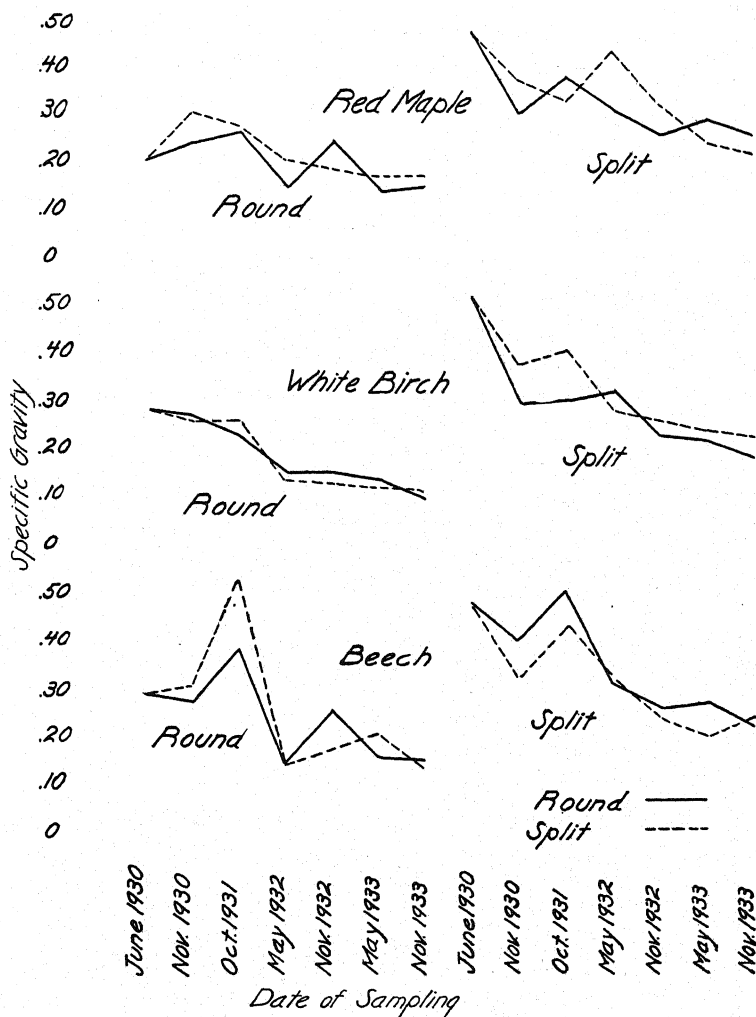


FIG. 1. Specific gravity of wood stored in the woods and in an open field as split and unsplit pieces of 4-foot cordwood.

ing a block of white ash, that in the area near the center of the tree the specific gravity was .65, while in the bark near the sapwood it was only .48. Later, Paul (7) studied the silvicultural aspects of specific gravity and found differences in trees of the same species growing only a rod apart. Similar factors may enter into the causation of the variable values given here.

The data presented in table 1, giving the B.T.U. values per pound, suggest that possibly the fungi have removed some components that may detract from or at least not contribute toward the heat value. By comparing figure

TABLE 1.—*Calorific value of wood stored in the woods and in an open field as split and unsplit pieces of cordwood. B.T.U. per pound of wood^a*

Date	Red maple				White birch				Beech			
	Woods		Open field		Woods		Open field		Woods		Open field	
	Round	Split	Round	Split	Round	Split	Round	Split	Round	Split	Round	Split
June 1930	8572.1	8551.3	8572.1	8551.3	8432.9	8389.7	8432.9	8389.7	8956.0	9080.5	8956.0	9080.5
Nov. 1930	8743.8	8193.7	8743.8	8193.7	8349.6	7689.3	8349.6	7689.3	8743.8	8780.4	8743.8	8780.4
May 1931 ^b	8610.4	8093.1	8378.2	7843.6	8845.1	8919.8
Oct. 1931	8750.3	8701.2	8746.9	8436.3	8012.9	7935.2	8136.7	7768.5	8873.4	8426.5	8916.5	7983.1
May 1932	7772.1	8258.7	8315.2	7783.5	7540.5	7711.3	7651.4	7658.4	7800.2	8112.3	7836.1	7924.7
Nov. 1932	8503.7	7637.1	7948.3	8043.1	7697.6	7673.0	7538.1	7646.1	8572.7	7758.3	7893.2	7793.5
May 1933	7546.1	7552.4	7648.9	7362.6	7640.8	7581.3	7371.2	7599.0	7753.6	7625.1	7813.9	7862.0
Nov. 1933	7369.4	7485.4	7428.6	7016.8	7101.8	7410.3	7169.2	7458.7	7720.6	7784.3	7700.5	7741.6

^a Converted from calories per gram by multiplying by the factor 1.8.

^b No samples were taken from the woods on this date.

1 with table 1 it is shown that the specific gravity decreased considerably faster than the fuel value on a per pound basis. This is contrary to previous opinions. Lehmann and Scheible (5) state . . . "Heretofore it has been generally held that losses in heat value which a given mass of wood may undergo through the action of fungi correspond to its losses in dry weight. . . ." The data they present do not support this view, so far as concerns pine sawdust. The results of this investigation likewise do not agree with the above cited opinion.

In order to interpret the results in a form applicable to general use, it was found necessary to determine the relative fuel value per cord. The first step was to learn the volume of wood per cord. This was done by first taking the approximate size of the sticks of each species and adopting the values for the respective sizes from a volume table such as given by Chapman (4, p. 131). It was then necessary to determine the pounds per cubic foot. This was done by taking the figure 62.5, the weight of 1 cu. ft. of water at 4° C., and multiplying it by the specific gravity. This, of course, disregards temperature, but the assumption was necessary in order to secure data applicable to the case. It was then possible to calculate the total absolute number of B.T.U. per cord. In all probability the method of sampling accounts for some of the variation in the values derived. This was partially compensated for by interpolation curves. The B.T.U. values per cord were converted to a percentage basis with the first or undecayed sample taken as 100 for each series. The results are shown in table 2.

A comparison of the figures given in table 2 for the samples from the woods in November, 1933, and those from the open field show that those in the woods have, with the exception of the split red maple and the round beech, lost more than the corresponding samples in the open field. The irregularity in the values given for these two series may be due to variation in the sampling, which was not smoothed out by the interpolation curves. Although the final values for the samples of split pieces in only half the cases are lower than the values for the corresponding samples of round pieces, in most of the pairings the values are lower for the samples of the split pieces. As shown in table 1, this relationship is maintained for the B.T.U. values per pound. Thus it is apparent that the samples of some of the split pieces have deteriorated more rapidly than the samples of the round pieces. Possibly the protective coating of bark on the round samples may have influenced this; although, as a rule, splitting is considered to permit rapid drying with the inference that deterioration through decay would be less. In that the dry and apparently sound part of the split pieces was intentionally avoided in the sampling, the comparison, in all probability, was not influenced by splitting of the sticks.

TABLE 2.—*Relative calorific value in absolute B.T.U. per cord for wood stored in the woods and in an open field as split and round 4-foot pieces of cordwood.* (Based upon an interpolation curve)

Date	Red maple				White birch				Beech			
	Woods		Open field		Woods		Open field		Woods		Open field	
	Round	Split	Round	Split	Round	Split	Round	Split	Round	Split	Round	Split
	100	100	100	100	100	100	100	100	100	100	100	100
June 1930	100	100	100	100	100	100	100	100	100	100	100	100
Nov. 1930	93	92	96	86	91	80	98	90	95	95	96	82
May 1931	85	84	86	71	85	69	91	81	90	91	92	70
Oct. 1931	81	70	83	65	77	54	86	73	83	85	89	67
May 1932	75	68	82	55	66	57	77	64	79	75	80	64
Nov. 1932	70	60	81	49	59	41	62	53	70	65	75	61
May 1933	67	54	80	44	56	39	50	48	60	56	65	58
Nov. 1933	66	48	76	40	32	34	38	39	47	44	46	46

As is shown in table 2, the rate of deterioration was about equal in both locations. The values are, however, somewhat lower in the woods than in the field. A comparison of the values for the round *vs.* split samples shows that the rate of deterioration is slightly less in the round form than when the wood has been split. The round red maple from the field shows a striking divergence from the split red maple in the same location.

A comparison of the values for each species shows the greatest loss near the end of the experiment for the round white birch. This is well illustrated in the values for the round samples from the woods. The values for the beech show about the same tendency as the white birch for the round samples; the values for the split samples, however, are erratic, especially at the beginning of the experiment.

Lehmann and Scheible (5) investigated the loss in calorific value of pine sawdust when inoculated with various fungi. They recorded a maximum loss of 42.9 per cent in the heating value of the sawdust after a 6-month period of incubation with *Armillaria mellea*. Other fungi gave lower readings. *Daedalea quercina* caused a loss of only 8.1 per cent. In the 3 species of hardwoods reported here no specific attempt was made to determine what fungi were affecting each sample of wood. Fruiting bodies of the following species were collected from the wood in both locations: *Polyporus hirsutus* (Wulf.) Fr., *P. pargamensis* Fr., *Panus stipticus* Bull., *Stereum purpureum* Pers., *Thelephora* sp., and *Daldinia* sp. In all probability other fungi were present, but were not in fruit. Vanin and Esupoff (9) also investigated the effect of decay on the calorific value of wood. They give a very good résumé of previous work done in this field in Russia. Their results show that wood loses from 21.4 to 72.3 per cent of its fuel value through decay. Unfortunately, the time interval was not given in the translation of their paper, and it, therefore, is impossible to compare their results with those reported here.

The values given in table 2 represent the absolute B.T.U. per cord, and do not take into consideration the amount of water present in the wood. As decay advances the moisture content increases considerably. Campbell (3) had stated that to heat a pound of water from the ordinary temperature of the room to the boiling point, evaporate it, and heat the steam to the temperature of the flue gases requires approximately 1200 B.T.U. Record (8) gives a similar figure. Consequently, the increase in moisture content reduces proportionally the practically useful calorific value of wood containing a given number of heat units.

When the total pounds of water in the wood is computed per cord and a correction applied, the useful B.T.U. values per cord can be calculated. This varies somewhat from the absolute B.T.U. values, especially in the 1932 and 1933 samples. Betts (1) gives a formula for comparing the price to

TABLE 3.—*Relative value per cord of the respective species stored in the woods and in an open field as split and round pieces of 4-foot cordwood. (Based upon interpolation curves)*

Date	Red maple				White birch				Beech			
	Woods		Open field		Woods		Open field		Woods		Open field	
	Round	Split	Round	Split	Round	Split	Round	Split	Round	Split	Round	Split
June 1930	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00	\$8.00
Nov. 1930	7.10	6.60	7.80	5.50	6.65	6.45	7.48	6.25	6.90	6.93	7.70	6.38
May 1931	6.20	5.28	7.55	4.10	5.35	4.95	6.60	4.65	5.75	5.85	7.05	4.90
Oct. 1931	5.40	4.15	7.25	3.40	4.10	3.60	5.30	3.40	4.68	4.68	5.80	3.70
May 1932	4.70	3.25	6.90	3.20	3.00	2.55	3.68	2.42	3.72	3.75	4.25	2.75
Nov. 1932	4.18	2.60	6.45	2.90	2.10	1.73	1.90	1.71	2.95	2.20	3.10	2.10
May 1933	3.70	2.20	5.90	2.30	1.35	1.20	1.20	1.36	2.33	1.70	2.35	1.75
Nov. 1933	3.64	2.13	5.13	1.43	.85	.94	1.06	1.32	1.87	1.66	1.99	1.73

be paid for wood in order to make it as economical to use as coal. This may be applied to the actual B.T.U. produced per cord in order to determine comparable values at any succeeding date after cutting in order to obtain a comparable number of heating units. Assuming the useful B.T.U. in the original samples as being worth \$8.00 per cord, and correcting for changes in water content and absolute B.T.U. in subsequent samples, the values secured are given in table 3.

As shown in table 3, the cordwood stored in the woods is appreciably lower in value than that stored in the open field. If this wood had been stored in the well-ventilated shed, its value would not have changed appreciably, inasmuch as the moisture content of the samples collected in June, 1930, was less than 30 per cent, which is considered near the minimum for fungal action. The loss is especially significant if we consider the round white birch stored in the woods. After 3 years' storage its value becomes only \$.85 per cord, while, under proper storage conditions, its value would still be approximately \$8.00.

SUMMARY

Assuming that all deterioration observed was caused by fungous decay, the effect of such decay on specific gravity and calorific value was measured in split and unsplit wood of 3 species, red maple, *Acer rubrum* L.; paper birch, *Betula papyrifera* Marsh.; and beech *Fagus grandifolia* Ehrh., in 2 types of situations over a period of 4 years.

Specific gravity of the wood when air-dried was found to be higher in split wood, presumably from trunks, than unsplit wood, presumably from branches.

As the specific gravity decreased, the calorific value of the wood, air-dried, also decreased somewhat, indicating a change in chemical composition.

The calorific value decreased somewhat faster in the woods than in an open field, faster in the split than in the unsplit form, and respectively more rapidly for the maple, beech, and birch in the round form.

Correcting the calorific value for water content, which increased with decay, the net or practically useful fuel value was found to decrease in 4 years as much as 89.4 per cent for the round white birch from the woods, and as little as 35.9 per cent for the round red maple from the open field. The decrease was as much as 50 per cent in less than 2 years in some of the birch wood.

UNIVERSITY OF MAINE,
ORONO, MAINE.

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APPEARANCE OF FOMES IGNIARIUS IN CULTURE

HENRY HOPP

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Wood-destroying fungi commonly produce a rather constant type of mycelial growth in culture. *Fomes igniarius* (L.) Gill., one of the most destructive of the xylophilous fungi, is peculiar in that it does not follow this general rule. Previous workers report that, with the exception of those obtained from species of *Populus*, sporophore-tissue cultures of this fungus isolated from different hosts are similar in appearance. The marked differences in appearance between the cultures obtained from *Populus tremuloides* Michx. and those obtained from *Alnus* sp. led Long and Harsch¹ to the belief that two different fungus species were concerned. Fritz² noted these differences but minimized their diagnostic value, since transfers from some of her cultures obtained from *Populus* did produce mycelium similar to that isolated from other hosts.

In the investigations cited the cultures were all started from sporophore tissue. In the study herein reported, the cultures were obtained from decayed wood. Three trees of *Populus tremuloides* infected with *Fomes igniarius* were used. One hundred and twenty small pieces of wood were taken from various places in the decayed trees and placed on Bacto malt agar in 31 Petri dishes. From these plantings, only 50 fungous cultures were obtained. A survey of the cultures showed that 2 types of mycelium were present. Forty-one cultures were of the type that, according to the literature, is obtained only from *Populus*. It was characterized by deep staining of the agar, and in color approximated mummy brown³. Surface mycelium was exceedingly sparse, and aerial mycelium was almost or entirely absent. This type of culture is referred to in this paper as the staining type. Nine cultures were of the type that, according to previous workers, is obtained only from sources other than *Populus*. The latter kind of culture, hereafter referred to as the bleaching type, was characterized by a more-or-less evident bleaching of the agar and by profuse aerial mycelium approximating antimony yellow to Dresden brown. In 5 of the 9 cultures, the agar was stained during the early growth of the mycelium, but was bleached during subsequent growth, while in the other 4 cultures, it was unstained from the beginning.

These results suggested that an effort to determine the cause for the 2 types of cultures might be worthwhile. To this end an attempt was made

¹ Long, W. H., and R. M. Harsch. Pure cultures of wood-rotting fungi on artificial media. Jour. Agr. Res. [U. S.] 12: 33-82. 1918.

² Fritz, C. W. Cultural criteria for the distinction of wood-destroying fungi. Roy. Soc. Canada, Proc. and Trans. III, 17 (Sect. V): 191-288. 1923.

³ The color nomenclature in this paper is that used by Ridgway, R. Color standards and color nomenclature. Washington, D. C. 1912.

to correlate the number of cultures of each type with (a) the individual host, (b) the zone of decay, and (c) the level along the longitudinal axis of the tree from which the inocula had been taken.

The decayed area was differentiated into several zones that formed concentrically arranged vertical cylinders within the stem of the tree. As viewed on a cross-section surface, the centermost cylinder was a zone of yellowish advanced decay. This was enclosed within a black border line, a narrow band encircling the advanced decay and separating it from the noninfected wood or from a dark zone of brown incipient decay. Within the advanced decay area, black lines were sometimes found. Their positions indicated that they were formerly border lines that persisted following the centrifugal extension of the advanced decay. Inocula were taken from the incipient decay, the black border line, the advanced decay, and the black lines contained within the advanced decay.

Inocula also were obtained at 4 levels along the longitudinal axis in the decayed portion of the trees. The different levels were (a) *above* the upper end of the black border line; (b) directly *at* the upper end of the black border line; (c) a short distance *below*, but within one meter, of the upper end of the black border line; and (d) near the *base* of the tree.

The data in table 1 indicate that the appearance of the cultures was independent of the source of the inocula. The type of culture was definitely not related either to the level in the tree or to the zone of decay from which the inocula were obtained. The production of only the staining type of culture from tree number 2 would suggest a host influence, except that inocula from trees number 1 and 3 gave both types of cultures.

TABLE 1.—*The number of staining and bleaching cultures obtained from Populus tremuloides decayed by Fomes igniarius*

Source of inoculum	Type of culture obtained	
	Staining	Bleaching
Tree number:		
1	13	4
2	19	0
3	9	5
Zone of decay:		
Incipient decay	0	1
Black border line	15	2
Advanced decay	23	6
Advanced decay and black lines therein	2	0
Black lines within advanced decay	1	0
Level in the tree:		
Above upper end of black border line	18	5
At upper end of black border line	2	0
Below upper end of black border line	14	3
Near base of tree	7	1

It was observed that, regardless of their origin, inocula usually produced the same type of culture when planted in the same Petri dish. Eighteen of the 31 Petri dishes contained more than one culture each. Both bleaching and staining types occurred in only 2 of these. Each of the other Petri dishes contained cultures of one type or the other, but not of both types. In 3 cases, duplicate inocula taken from within less than 2 centimeters of each other in the advanced decay produced different types of cultures when they were planted on agar in separate Petri dishes.

Thirty-two transplantings in 8 Petri dishes were made from 2 bleaching and 2 staining cultures, so that within a single Petri dish 2 inocula from each type of culture were grown. The subcultures were of the same 2 types, and, interestingly enough, inocula taken from the staining cultures produced both staining and bleaching subcultures. The cultures obtained in one Petri dish were generally similar in appearance irrespective of their source, although inocula taken from the same source, but grown in separate Petri dishes, produced in 6 cases subcultures of different types. Multiply transfers from 10 additional cultures confirmed the observation that growth of both types can be secured from the same mycelium when it is placed on agar in different Petri dishes.

It was recognized that tissue cultures obtained from decayed wood might contain more than an individual fungus. In order to isolate individual organisms, single hyphal tips, cut from mycelium growing on non-nutrient agar, were cultured in separate Petri dishes. Multiply transfers from these cultures showed that mycelium originating from a single hyphal tip can produce both staining and bleaching types of growth. Single-spore cultures were then studied in order to determine whether they would give variations in type when subcultured. Accordingly, 13 spores from a sporophore of *Fomes igniarius* produced on *Populus tremuloides* were isolated and placed on malt agar. Among the resulting cultures were staining and bleaching types; and multiply subcultures from each of these were also of both types.

These data indicate that *Fomes igniarius* isolated from wood of *Populus tremuloides* may produce 2 types of mycelial growth on malt agar. Homothallic cultures obtained from mycelium growing in wood and from spores also produced both types of growth. The type of mycelial growth in cultures obtained from *Populus* has little to do with the source of the inoculum but is related to some other factor yet unknown. The conditions of the culturing, however, might be of some importance in determining the type of resultant culture. No indication was observed of a genetic difference in the mycelium found in the bleaching and staining cultures isolated from *Populus*.

PHYTOPATHOLOGICAL NOTES

Spotted Wilt of Garden Pea.—In October, 1934, certain pea plants (*Pisum sativum* L.) in one of the University of Wisconsin greenhouses were affected by a streaking of stem and a spotted brown necrosis of leaves that often resulted in their premature death. Inoculation of expressed juice from these disease plants into tobacco, tomato, aster, nasturtium, *Nicotiana glutinosa* L., *Datura stramonium* L., and *Emilia sagittata* DC., produced symptoms identical with those associated with the virus of spotted wilt of tomato. In 12 out of 18 Yellow Admiral pea plants inoculated by rubbing with juice from spotted-wilt-infected Calla lily leaves (virus from M. W. Gardner, California) and in 4 out of 16 plants inoculated by the tissue-insertion method of Stubbs,¹ symptoms developed, identical with the naturally occurring streak. Ten noninoculated plants remained healthy. Carborundum powder, used as an abrasive, greatly facilitated infection. Pea plants of varying stages of growth were infected by the rubbing method, but the highest percentage of infection occurred on seedlings inoculated soon after emergence, and before the trifoliate leaves had unfolded. From 105 Yellow Admiral, Alaska, and Alderman plants, inoculated by the rubbing method, 33 showed typical symptoms and the 3 varieties appeared to be about equally susceptible.

Infective thrips (*Thrips tabaci* Lind.) were transferred to healthy peas in one series from nasturtiums infected with the pea-streak virus, and in another series from nasturtiums infected with the spotted-wilt virus. Symptoms developing in both series appeared to be identical and typical of the original pea-streak disease.

Of 192 plants so treated 43 showed symptoms. Results were positive in each of 6 attempts to recover the virus from peas thus inoculated. When infective thrips were caged on 27 pea blossoms, 17 set pods and symptoms appeared on 6, from which the virus was readily recovered.

Under these experimental conditions the first symptoms usually appear 7 to 20 days after inoculation as necrotic streaks on the stem and as spot or vein necrosis on young leaves. Frequently, however, the initial symptom appears as a distinct necrotic spot on the artificially inoculated leaves, or in association with thrip-feeding injury when the virus is transmitted by that vector. The stem streak is purplish or bluish brown and may extend only a little beyond the point of inception or over the entire length of the stem. A histological examination of infected stems showed general necrosis of parenchymatous cells, including the phloem. Unilateral development of affected parts is frequent. Occasionally a mottled pattern develops on leaves

¹Stubbs, M. W. Viroses of the garden pea, *Pisum sativum*. (Abs.) Phytopath. 26: 108-109. 1936.

infected when young. Local infections of immature pods may appear as small, circular necrotic spots associated with the thrips-feeding marks, and necrosis may later extend into irregular, wavy, concentric patterns over most of the surface. Systemic infection may result also in necrotic or concentric patterns on the pods; however, if these are systemically or locally infected when very young, they often become stunted, distorted, and collapsed. The seeds occasionally show necrotic spots or patterns (Fig. 1).

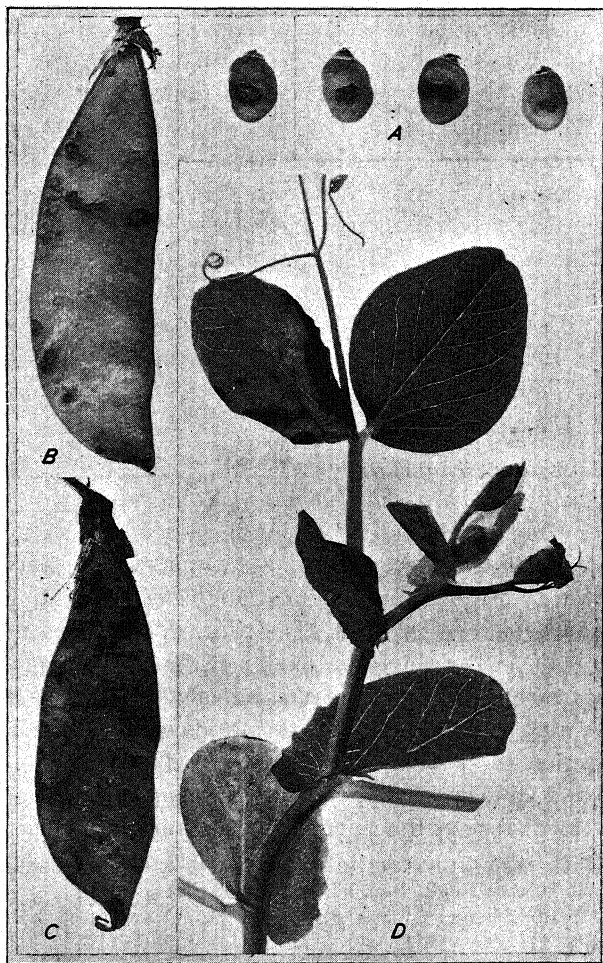


FIG. 1. Symptoms of spotted wilt on pea. A. Necrotic lesions on naturally infected pea seed. B-C. Pods, naturally infected in the field, showing mild and severe symptoms. D. Artificially inoculated pea plant showing severe necrosis of growing point, stem, and leaflets and unilateral development of affected parts.

Linford noted a streak of peas, widely distributed in the United States, in 1928.² He later produced similar symptoms by transfer of thrips to pea from *Emilia sagittata* affected with pineapple yellow spot,³ and found pea-streak plants near Honolulu, Hawaii, naturally infected with this virus. He suggested that this or a related virus caused pea streak in the United States. Stubbs produced a streak symptom in pea by inoculation with tobacco ring-spot virus, and Zaumeyer and Wade^{4, 5} produced a streak in pea by inoculation with viruses from white sweet clover, sweet clover, and alfalfa. The demonstration that the tomato spotted-wilt virus causes a streak of pea is of peculiar interest inasmuch as the virus has much in common with that of pineapple yellow spot in the way of insect vector, incubation period, and almost identical symptoms on *Emilia sagittata*.⁶ In view of the fact that the spotted-wilt virus is widely distributed it is undoubtedly commonly a causal factor in pea streak. Symptoms similar to those described in the greenhouse were observed in the field at Madison, Wisconsin, in the summer of 1935 on pea plants artificially inoculated with the spotted-wilt virus. An epidemic of pea streak, which developed in two widely separated pea nurseries at Madison in the same year was due in part to spotted wilt, inasmuch as the virus was recovered from badly diseased plants.—OTIS C. WHIPPLE, University of Wisconsin, Madison, Wis.

Injury to Greenhouse Tomatoes as a Result of a Combined Infection with the Viruses Causing Tomato and Cucumber Mosaic.—During the spring of 1935, two cases came to the writers' attention in which greenhouse tomatoes in Ohio and Colorado were severely damaged by a virosis caused by a combined infection with ordinary tomato mosaic (*tobacco virus 1*, Johnson) and cucumber mosaic (*cucumber virus 1*, Doolittle). The tomato is known to be susceptible to both, either singly or combined, but the combined infection, apparently, has rarely assumed economic importance. In both cases, however, the losses were estimated at some thousands of dollars and it is evident that such infection occasionally may prove a definite menace to the crop. The Colorado and Ohio specimens were alike in appearance, and the writers also observed the disease as it occurred in the Ohio greenhouse.

² Linford, M. B. Pea diseases in the United States in 1928. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. Sup. 67. 1929. (Mimeographed.)

³ Linford, M. B. Streak, a virus disease of peas transmitted by *Thrips tabaci*. (Abs.) Phytopath. 21: 999. 1931.

⁴ Zaumeyer, W. J., and B. L. Wade. Mosaic diseases affecting different legumes in relation to beans and pea. Phytopath. 23: 562-564. 1933.

⁵ Zaumeyer, W. J., and B. L. Wade. A pea streak caused by alfalfa mosaic. (Abs.) Phytopath. 26: 114. 1936.

⁶ Linford, M. B. Transmission of the pineapple yellow-spot virus by *Thrips tabaci*. Phytopath. 22: 301-324. 1932.

In this latter case the tomatoes had been planted in November and, when observed on May 30, were abnormally short (3-4 feet) and compact. The leaves just below the growing point had a peculiar upright, bushy habit of growth that was due partly to very short internodes and partly to an abnormally twisted and erect growth of the petioles (Fig. 1). The young leaflets

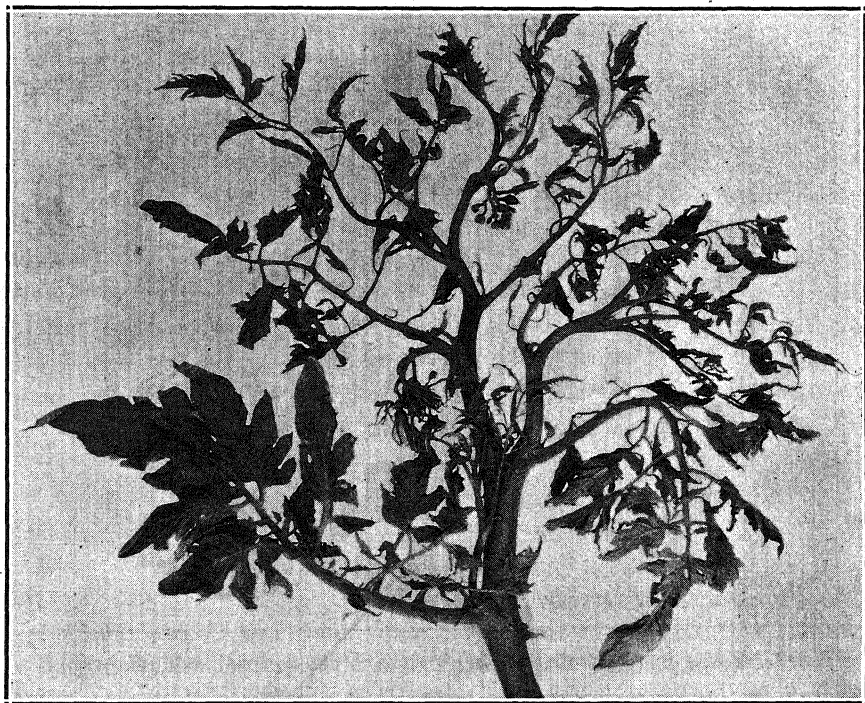


FIG. 1. Upper portion of greenhouse tomato plant showing malformations of foliage and peculiar habit of growth due to combined infection with the viruses of cucumber and tomato mosaic.

were curled and distorted and occasionally showed the filiform "shoestring" malformations that often occur on tomatoes infected with cucumber mosaic. These leaflets were thick and showed a pronounced yellowing of the veins. The older leaflets generally were rolled sharply upward at the margins and carried a mild, yellow-green mottling similar to that of ordinary tomato mosaic. Many of the leaves, however, were of a peculiar greenish purple, particularly along the veins and on the under surface of the leaf. Some leaves also showed large, yellow spots of a bleached appearance accompanied by a russet-color necrosis bordering the larger veins. This necrosis, however, was not sufficiently severe to cause serious injury to the leaf. The blossoms were commonly malformed and abortive; but the plants had produced some

fruit, which was deeply ridged and, when small, showed a characteristic pointed protuberance at the blossom end (Fig. 2). These symptoms are not characteristic of either of the viruses alone and were new to the writers.

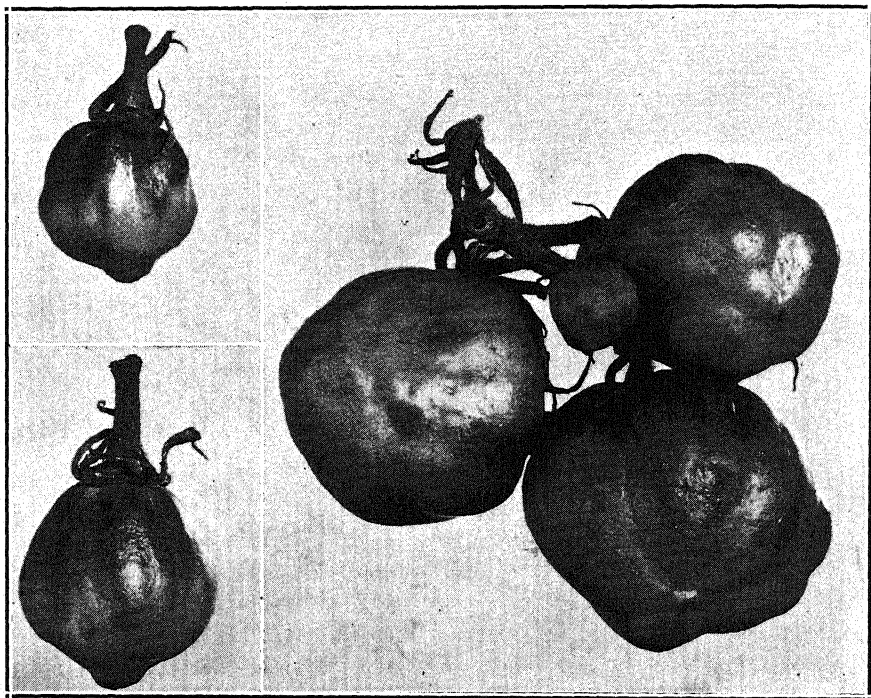


FIG. 2. Young tomato fruits showing irregular, ridged growth and pointed protuberance at blossom-end caused by a combined infection with the viruses of cucumber and tomato mosaic.

Inoculations were made by the senior writer to tomato, cucumber, and tobacco, using diseased tomato material from both Colorado and Ohio. The inoculated tomato plants developed the peculiar leaf color and necrosis already described, and the later growth showed leaf malformations similar to those noted in the greenhouse. Since the cucumber is not susceptible to tobacco mosaic, the cucumber virus was readily separated from the accompanying tobacco virus by direct inoculations to cucumber. On both cucumber and tobacco the cucumber mosaic virus produced symptoms characteristic of cucumber virus 1. The tobacco mosaic virus was separated from the cucumber virus by heating the extracted juices of plants carrying both viruses to 82° C. On tobacco and tomato, the remaining virus appeared identical with tobacco virus 1. Inoculations to *Datura stramonium* indicated that the common latent mosaic of potato was not a factor in the disease in question.

Owing to its rapid dissemination by pruning and handling, tomato mosaic is so prevalent on greenhouse tomatoes that the introduction of another virus (such as cucumber mosaic) is almost certain to result in combined infection. Cucumber mosaic, however, is less readily transmitted to tomatoes by mechanical means, and aphids apparently are responsible for most of its secondary dissemination. This probably accounts for the rarity of losses from the combined infection, since aphids are effectively controlled in most tomato greenhouses. In Ohio, the cucumber mosaic infection was traced to a near-by field of muskmelon, and, in Colorado, it apparently originated on cucumbers in another section of the greenhouse. In both instances aphids apparently were present in considerable numbers. In the fall of 1935, the combined infection appeared on 2 plants in the greenhouse at Wooster, Ohio, evidently as a result of aphids carrying the cucumber virus from cucumbers in the field. A few infected plants also were noted in another Ohio greenhouse, but in both instances periodic fumigation was practiced and there was no secondary spread of the disease.—S. P. DOOLITTLE, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and L. J. ALEXANDER, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster, Ohio.

A Method of Separating the Teliospores of Cronartium Ribicola.—The teliospores of *Cronartium ribicola* Fischer, the blister rust of five-needle white pines, are borne in slender hair-like columns (Fig. 1, A) on the under surface of the leaves of various *Ribes* species. Studies now in progress, involving a quantitative determination of the teliospore-production capacities of several *Ribes* species, necessitated finding a method of separating the spores from the telial columns, in order that rapid and accurate counts of spores per column could be secured. The primary obstacle confronting simple separation of the spores is a matrix that binds the teliospores (Fig. 1, B) firmly together in compact columns. So inert is this matrix that ordinary hydrolytic agents do not render it soluble if used in concentrations sufficiently dilute not to be corrosive to the teliospores. Mechanical maceration following hydrolysis merely resulted in breaking the spores transversely without separating them from the matrix. The ideal reagent sought was one that would dissolve out the matrix but leave the column intact to facilitate mounting singly on a slide, permitting complete separation of the teliospores (Fig. 1, C) by rolling the column between slide and cover slip. It finally was discovered that a 1-Normal nitric acid solution, if properly manipulated, had all the desired features. The solubility of the matrix in nitric acid indicates that it possibly contains some pectic substance. Confirmatory evidence of this has been obtained when the solution, in which a

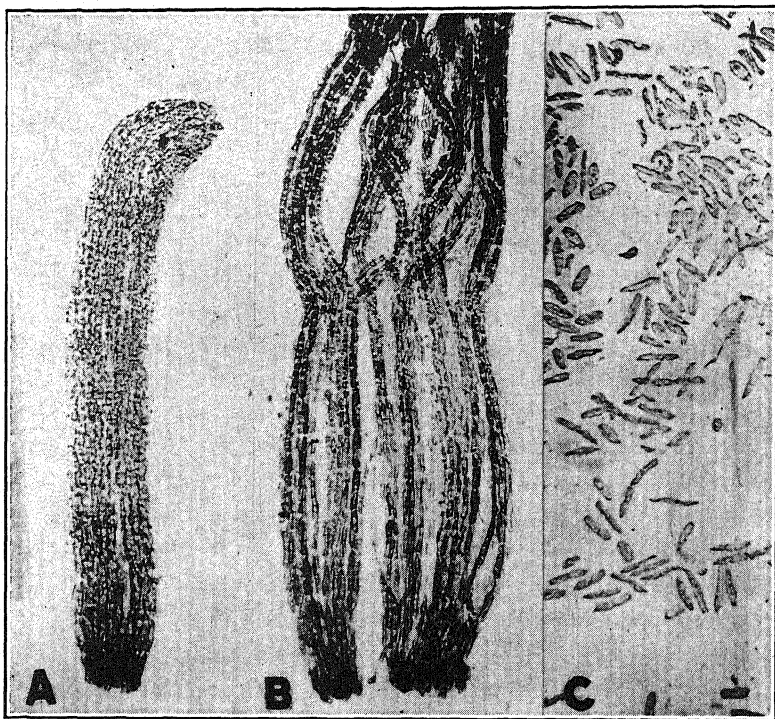


FIG. 1. A. Entire mature column of *Cronartium ribicola* after nitric acid treatment. The general appearance of the column is slightly affected by the treatment. B. Longitudinal splitting of the teliospore chains. C. Complete separation of the teliospores. All $\times 90$.

large volume of telial columns had been treated, was further treated with concentrated nitric acid and then yielded crystals of mucic acid.

The following procedure has given consistent results in routine laboratory practice of separating teliospores. Fragments of fresh or dried ribes leaves bearing telial infection are placed in a receptacle and covered with 1-Normal nitric acid. The mixture is boiled vigorously over a flame until the columns begin to become detached from the leaf surface and take on a bleached appearance. Cold water is quickly added to halt the reaction. More columns are removed by stirring with a glass rod, also used to remove the leaf fragments from the solution. The columns settle rapidly out of suspension, permitting the water to be decanted off with no great loss of material. Four or 5 additional changes of water suffice to wash out the remaining acid. The use of alkalis to neutralize to acid has no advantage and usually results in discolored spores. Nearly all of the remaining water can be removed from the columns by transferring to a small vial and draw-

ing it out with a pipette. The columns are then ready for mounting in glycerine or glycerine jelly or they can be kept in glycerine to which some eosine or erythrosin has been added.

If correctly treated the entire mature column should appear as illustrated in figure 1, A, and under slight pressure should split as shown in figure 1, B. Further pressure and movement of the cover slip will break up the chains of spores and complete the separation (Fig. 1, C). The method has been tried and used with success on *C. ribicola* from 10 *Ribes* species native to western United States and on *C. occidentale* Hedgcock, Bethel and Hunt, the pinyon blister rust, from 2 species of *ribes* native to its range.—
R. K. PIERSON.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
PORTLAND, OREGON.

(In cooperation with Emergency Conservation Work and the School of Forestry, University of Idaho.)

A Preliminary Note on Sexuality in Ceratostomella ulmi.—In 1932 Buisman¹ reported that the Dutch elm disease fungus *Graphium ulmi* had an ascomycetous stage and was heterothallic. She named it *Ceratostomella ulmi*. Its perithecia have not been found on diseased trees in this country, but their importance in the life history suggested an investigation of sexuality in the fungus and of the distribution of races of the fungus in the United States.

Mass isolates secured from the Dutch Elm Disease Laboratory at Morristown, New Jersey, and approximately 2,000 monoconidial isolates from them were tested for formation of perithecia by growing them singly and in combinations on sterilized elm twigs. All monospore isolations were made with a micromanipulator or microloop.²

Monoconidial isolates did not form perithecia, whereas crosses of some of them from different mass isolates did. In a few cases crosses of monoconidial isolates from the same mass isolate also produced perithecia. In such cases it was later determined that both plus and minus races were present in the original isolate.

Monoascope isolates from perithecia resulting from pairing monoconidial isolates have repeatedly produced the *Graphium* stage in culture. An American elm, inoculated under quarantine conditions with a suspension

¹ Buisman, Christine. *Ceratostomella ulmi*. De geslachtelijke vorm van *Graphium ulmi* Schwarz. Tijdschr. Plantenz. 38: 1-5. 1932.

² Dunn, M. S. The microloop. A rapid method for isolating single spores. Phytopath. 14: 338-341. 1924.

made from a monoascospore isolate, developed typical symptoms of the Dutch elm disease after two weeks. The Graphium stage was recovered from the tree.

Monoascospore isolates did not form perithecia, but, when paired, perithecia developed in some crosses within 9 to 42 days. Some crosses produced abundant perithecia; others, only a few. The results of crosses among 14 monoascospore isolates are given in table 1.

The perithecia secured by the writer agree morphologically with those described by Buisman. She, apparently, observed no asci. The writer observed asci in different stages of development by killing the material in 75 per cent chrom-acetic acid and mounting in lacto-phenol.³ The asci are hyaline, clavate, tapering sharply to the point of attachment, and measure 8.75–15 μ by 3.1–6.8 μ averaging 10.9 μ by 5.36 μ at the widest portion. The number of spores in an ascus could not readily be determined; in 2 cases it was thought that 8 were observed.

Isolates from diseased trees in the United States were grown in combination with races of *Ceratostomella ulmi* designated as plus or minus, on the basis of their consistent reaction in previous experiments. Both plus and minus races were found among the isolates from New Jersey, New York, Ohio, and Virginia. Minus races alone were found in Indiana and Connecticut, but only a few isolates from these states were obtained for experimental work. Both plus and minus races were found in one tree in New Jersey and in another in Ohio. Both races were found in close proximity in New York and New Jersey.

TABLE 1.—Results of duplicate combinations of 14 monoascospore isolates of *Ceratostomella ulmi*.^{a, b}

	No. 5	No. 8	No. 10	No. 11	No. 12	No. 13	No. 14
No. 1	P	—	—	P	P	P	P
No. 2	P	—	P	P	O	O	P
No. 3	P	—	—	—	O	P	—
No. 4	P	O	P	P	P	P	P
No. 6	P	P	P ^c	P	O	—	P
No. 7	P	—	P	—	O	P	P
No. 9	P	P	P	—	P	P	P

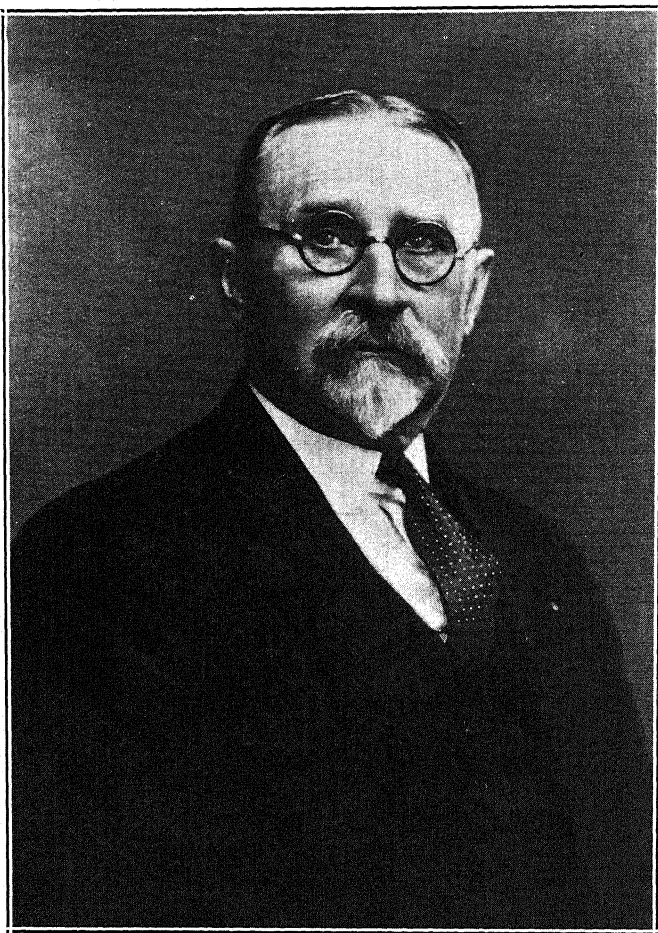
^a P, perithecia; O, no perithecia; —, no cross made.

^b No perithecia were formed when numbers 5, 8, 10, 11, 12, 13, and 14 were paired with one another nor when 1, 2, 3, 4, 6, 7 and 9 were paired with one another.

^c Not duplicated.

³ Rawlins, P. E. *Phytopathological and Botanical Research Methods*. John Wiley & Sons, New York, 1933.

It has not been possible to differentiate races of the fungus morphologically or culturally except on the basis of their ability to form perithecia. Variants or sectors, strikingly different from the parent culture, frequently are produced by both monoconidial and monoascospore isolates. In 75 variants from one monoascospore isolate, the plus or minus characteristic either remained unchanged from that of the parent culture or the ability to form perithecia when crossed with certain test isolates was lost, the production of conidia, however, appeared to remain unchanged.—ROGER U. SWINGLE, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Ohio Agricultural Experiment Station, Wooster, Ohio.



SAMUEL HENRY ESSARY

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SAMUEL HENRY ESSARY

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J. O. ANDERSON

Professor Samuel Henry Essary passed away suddenly on April 28, 1935, from a heart attack. At the time of his death he was Botanist of the Tennessee Agricultural Experiment Station and had served the institution since 1904. His researches in the field of botany and plant pathology were extensive in character but thorough, and consequently were well known by professional workers, not only in these fields but also in allied groups. He was equally well known for his comprehensive knowledge of the flora and geography of the Southern Appalachians. By continuously applying his knowledge of scientific principles to practical phases of plant improvement, he made notable contributions to the agriculture of the Southeast.

Born in West Tennessee, September 11, 1870, a descendant of pioneer stock, he grew up in an environment of nature on the home farm and always retained his love for the outdoors and agriculture. Completing the elementary schools, he entered the University of Tennessee, graduating in 1897. From 1899 to 1902 he taught in LaGrange College in Missouri, and from 1902 to 1904 in Brenau College at Gainesville, Georgia. In 1904 he was appointed instructor in the Botany Department of the University of Tennessee, and Assistant Botanist of the Experiment Station. He took his Master's degree at the same institution in 1907, and also spent some time in study at the University of Wisconsin. From 1912 to 1919 he was Associate Botanist of the Experiment Station, and became Botanist in 1919.

Always a public spirited man, he belonged to numerous organizations. He took an active interest in Masonic affairs, being a member of Kerbel Temple A.A.O.N.M.S. of Knoxville, Tennessee, and was a member of the Baptist denomination. He was a fellow of the American Association for the Advancement of Science, a charter member of the American Phytopathological Society, a member of the American Genetics Association, American Society of Agronomy, Tennessee Academy of Science, and other local scientific societies, and belonged to Phi Kappa Phi, Alpha Zeta, and the S.A.E. social fraternity.

Early in his career he was associated with the late S. M. Bain in developing anthracnose-resistant clover and carried the work steadily forward after Professor Bain's death in 1918. Improving a native lespedeza by careful selection, he produced "Tennessee 76," a variety widely grown in Tennessee and other states. His Tennessee Red and Tennessee Pink tomatoes were developed for wilt resistance and thus filled a sorely felt want. He made many selections of special regional value and tested many crops and native plants for their relative merit and their possible economic value. Forage crops and legumes seemed to hold his first interest, although he worked successfully with reference to varietal adaptation and disease control in other groups. During the past several years he had done considerable work with the selection and breeding of cotton.

While a student he became interested in the extensive region now composing the Great Smoky Mountains National Park. At that time it was a vast wilderness of deep gorges and rugged peaks, heavily timbered and abounding in wild life of all kinds. Professor Essary worked untiringly for the preservation of these natural beauties, and many of his excellent and unusual photographs which were anonymously published throughout the world were used in calling the attention of the public to the scenic value of this area. He blazed many trails through this virgin forest that others might follow safely, and spent many days botanizing in the region. His amiability and his desirability as a comrade in the woods can be vouched for by the many people who have spent days and nights with him over hard and tiring trails. His botanical collections for 40 years were very extensive and his intimate knowledge of the plants of the region was unsurpassed, due largely to his ability to cover great distances and his unusual powers of observation.

Usually called "Professor" Essary, he officially taught only a short while, but devoted many hours each day to research. Although a very industrious man, he always found time to help others with their particular problems and influenced numerous students to take up the field of Botany. Of an exceedingly kindly disposition and indifferent to his own personal wants and advancement, his greatest pleasure was to see the science for which he had devoted his own life advanced by the accomplishments of those younger men in whom he was especially interested.

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THE EFFECT OF CERTAIN CULTURAL AND HANDLING PRACTICES ON THE RESISTANCE OF APPLES TO *PENICILLIUM EXPANSUM*¹

KENNETH F. BAKER² AND F. D. HEALD³

(Accepted for publication Oct. 21, 1935)

The success of the Washington apple industry is due in part to its practice of placing on the market a constant supply of uniformly high quality fruit in convenient containers. Among the important factors of culture and handling that determine the quality and condition of fruit are the application of orchard fertilizers, the time of harvest, and the length of the storage period. To maintain a high yield the application of fertilizer has become standard practice. Since grade of apples is determined largely by the color, they are frequently left on the tree past prime maturity in order to meet the requirements for extra-fancy fruit. The commercial crop is held in cold storage for protracted periods, approximately half being stored locally and half at eastern terminals. The storage life of the three principal varieties of the State is as follows: the Jonathan crop is consumed largely by the last of January, the Delicious by March 1, and Winesap apples are available until about July 1. Fungous decay is augmented by this prolonged storage, and assumes great importance because of the high unit value of the crop and the necessity of preserving a favorable reputation. It is recognized by the members of the industry that there is a distinct correlation in a given variety between the length of the storage period and the percentage of decay.

Blue-mold decay, caused by *Penicillium expansum* Link, is the most important storage rot of apples. A conservative estimate⁴ places the annual loss for the apples *actually decayed* by this organism in the Washington crop at about \$448,000. The accompanying losses from such items as cost of repacking, lowering of price level, and dumping of fruit are far greater, but are not assessable.

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² Formerly Research Assistant in Plant Pathology, Washington Agricultural Experiment Station, Pullman, Washington.

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⁴ Computed from the average annual value (\$27,988,400) of the commercial apple crop for the 5-year period, 1925-1929 (30), and the average 1.6 per cent of fruit that showed blue-mold decay in the 6.3 per cent of the total car shipments inspected at eastern terminals in the same period (3, 15, 28).

Penicillium expansum is able to infect apples through injuries of the epidermis and through lenticels, the calyx basin and canal, the stem, and necrotic areas. With proper handling of the varieties grown in Washington only the first two infection courts are of commercial importance. The ability of the fruit to withstand infection at lenticels, the stem, or the calyx basin or canal may be considered as external resistance. After infection has occurred, the rate of advance of the decayed area is influenced by the congeniality of the tissue as a substratum (*i.e.*, the degree of internal resistance). Much of the published work on resistance of apples to decay has dealt with the internal type, but some papers (3, 4, 6, 19, 15, 22, 31) have considered external resistance.

The purpose of these investigations was to determine the effect of application of orchard fertilizers, of maturity at the time of picking, of the length of the storage period, and of fruit variety on the incidence of lenticel infections and the rate of advance of the blue-mold decay of apples.

MATERIALS AND METHODS

The fruit used in these studies was obtained from the Experiment Station orchard in Wenatchee. It was sorted in the orchard for size (ranged from 113 to 150) and extra-fancy grade, only fruit free from injuries of the epidermis being used. It was packed without being washed, shipped at once to Pullman, and placed in cold storage ($0^{\circ} \pm 0.3^{\circ}$ C., unless otherwise specified).

The following method of inoculation of punctures was used in the series for determination of internal resistance. The end of a stick was cut so as to leave a peg projecting from the shoulder formed by the cut end; this tool gave artificial stem punctures in the fruit uniformly 4 mm. in diameter and of the same depth. Each apple was punctured at 3 points equidistant about its greatest circumference by this simple wooden device. The fruit was then immersed in an aqueous suspension of spores of the strain of *Penicillium expansum* employed in previous work (4, 5). Approximately the same amount of inoculum was employed each time in a uniform amount of water, and the fruit was rotated in it for a uniform period of time. The apples were then wrapped and packed in the usual way. Each spore suspension was made up as needed and was used for the inoculation of a single box of fruit.

Most of the studies on internal resistance of apples to fungous decay have been conducted by careful inoculation of a comparatively few specimens. Therefore, a justification should be given for the simple and rapid, though somewhat crude, method described above. The major objections to such a procedure are the possibility of contamination and the lack of complete uniformity of the amount of inoculum. Due to the characteristic

inhibitory action of *Penicillium expansum* on other apple-rotting fungi and its tolerance of their staling products (23), its relatively rapid growth in apples at cold storage temperatures, the high population of spores employed, and the fact that some fungi do not cause decay at 0° C., there was very little contamination in the series. In the 9294 punctures so inoculated only 23 (0.25 per cent) showed contamination. Such a condition is in line with the findings of Machacek (23) in the commercial storages of Montreal and of the writers in the commercial storages of Washington. The principal contaminant encountered in these studies was *Botrytis cinerea* Pers. Apples infected by organisms other than blue mold were not entered in the tabulated data. The same technique will not apply to other apple-rotting fungi. For example, a similar lot of fruit immersed in a suspension of spores of *Gloeosporium perennans* Zeller and Childs gave almost complete infection with *Penicillium expansum* by the spores rinsed from the fruit surface into the punctures.

In the 9294 punctures successful inoculation with *Penicillium expansum* was effected by this method in 95.5 per cent of the cases. In the computation of the probable error of the mean in each of the series, the number of successful inoculations was taken as n , the value of which can be determined from the data presented on the total number of punctures and the percentage of punctures decayed. The diameter of the decayed areas, the index found by Gregory and Horne (13) to be the best measure of the internal resistance, was taken as the criterion of susceptibility to the advance of decay.

The wide differences previously observed (4) in the rate of advance of *Penicillium expansum* in apples of uniform size, maturity, and history of development, with a definite amount of inoculum placed in punctures of uniform size, and held in storage of constant temperature and humidity, has been observed further in these studies. The difference in apples, and in punctures in the same apple, demand a high population in order to determine the true susceptibility. It is felt that a large population handled in the way outlined will give a truer picture of the mean susceptibility than will a low population inoculated and handled with more care. The usual formulae were used for computation of the probable error of the mean, probable error of the average of averages, and the probable error of the difference.

The following method of inoculation was employed to give a measure of the relative susceptibility of a series of apples to lenticel infection (*i.e.*, the mean number of susceptible lenticels per apple). Jonathan apples obtained from the Experiment Station orchard in Pullman and inoculated with *Penicillium expansum* by the method just outlined were held at room temperature until nearly decayed and then placed in cold storage. The

decayed tissue was stirred in a container, and enough of the juice decanted to leave a fairly firm paste. Some of this was coated thinly over each apple; the fruit was wrapped at once in a double thickness of oiled apple wrap, packed, and returned to storage. A fresh batch of this inoculum was made up each time a lot of fruit was inoculated. The effectiveness and accuracy of this method of determining the number of susceptible lenticels per apple has been discussed in an earlier publication (4). It should be noted that the results are in agreement with those previously obtained by dipping the fruit in a spore suspension before storage (4). As already reported, the difference is one of degree rather than type, and gives data qualitatively comparable to those obtained under commercial conditions. In the 1085 apples inoculated in this study only 3 (0.28 per cent) showed contamination and, in these cases, at one lenticel per apple. The contaminant was *Botrytis cinerea*.

EFFECT ON RESISTANCE OF THE APPLICATION OF ORCHARD FERTILIZERS

The literature concerning the effect of fertilizer application on the resistance of fruit to fungous decay is rather unsatisfactory. In some studies the fungi causing decay have not been determined, in others no distinction has been made between external and internal resistance of the fruit. Most of the investigations have dealt with weaker parasites than *Penicillium expansum*. This problem has been studied in England for a number of years in connection with investigations of the correlation of chemical composition of the fruit and resistance to various fungi. There has been a paucity of research on this subject in other countries.

There is rather general agreement that fruit from soils high in available nitrogen has a higher nitrogen content than fruit from check plots (1, 17, p. 284-289, 18, p. 292-300, 11, 24, 26), although there are some minor inconsistencies. Plagge (26) found that this increased nitrogen content was principally in the noncolloidal fraction, and that the decrease of this fraction in storage was more rapid in fruit from check plots than from those receiving nitrogen. The condition of high nitrogen content of the fruit has been shown to be correlated generally with high susceptibility to decay by such fungi as *Fusarium fructigenum* Fr., *Cytosporina ludibunda* Sacc., and *Diaporthe pernicioso* E. & E. March. (16, p. 135-139, 17, p. 284-289, 18, p. 292-300, 20), but some exceptions have been noted (19). Gourley and Hopkins (11) did not find a correlation between application of nitrogenous fertilizers to the soil and the amount of storage decay (unspecified cause) in the fruit produced. Carter (8) has given a possible explanation of these inconsistent results. He showed that *Pleospora herbarum* Pers., *Fusarium fructigenum*, *Polyopeus* sp., and *Alternaria* sp. varied with the pH in their response to the nitrogen content of the fruit. Apples of low acidity dis-

played decreasing susceptibility with an increased nitrogen content. The effect of the application of a given salt is conditioned further by the supply of it already available in the soil.

The published data on the effect of other fertilizers on the resistance of apples to fungous decay is less definite and consistent.

The effect of applications of nitrogen, potash, and phosphorus fertilizers, and their various combinations on the incidence of lenticel infection and the rate of advance of *Penicillium expansum* in the tissue, was investigated to some extent. Prime maturity Jonathan apples were obtained from the Experiment Station fertilizer plots in East Wenatchee. The fertilizers used were ammonium sulphate (N), superphosphate (P), and potassium chloride (K). Six pounds of superphosphate and 5 pounds of each of the other components were used annually per tree. The applications began in 1927, with the exception of NP and NK plots, which were started in 1928. The check plot had received no fertilizer since 1928. The fruit was picked, packed, and shipped at once to Pullman, where it was placed in storage.

The fruit of the 1930 series was punctured and inoculated on January 27, 1931, in the manner described and was held in storage at -0.5° to 1° C. Measurements of the decayed areas on March 18, after an interval of 50 days, gave the results shown in table 1.

TABLE 1.—Mean diameter of 50-day-old decayed areas in Jonathan apples from various fertilizer plots. 1930 series

Fertilizer treatment	Total punctures	Percentage punctures decayed	Mean diameter (mm.)
N	222	96.85	29.637 \pm .282
P	249	100.00	36.241 \pm .349
K	222	99.55	29.880 \pm .258
NPK	450	97.78	32.880 \pm .176
Check	201	98.01	24.215 \pm .303

The fruit of the 1933 test was picked at prime maturity on September 27–28 and was punctured and inoculated on December 17, 1933. The size of the lesions on January 16 (after 30 days) and February 15, 1934 (after 60 days), is given in table 2.

The results of the series of these 2 years justify no further conclusion than that the addition of fertilizers to the trees increased slightly the rate with which *Penicillium expansum* decayed fruit tissue. The application of any of these 3 salts alone or in combination decreased the internal resistance, but the degree of the effect was rather inconsistent throughout.

Organisms poorly adapted to the conditions of growth in the tissue of apples would be more strongly affected by alteration of chemical composition

TABLE 2.—Mean diameter of decayed areas in Jonathan apples from various fertilizer plots 30 and 60 days after inoculation. 1933 series

Fertilizer treatment	Total punctures	Examined after 30 days		Examined after 60 days	
		Percentage punctures decayed	Mean diameter (mm.)	Percentage punctures decayed	Mean diameter (mm.)
N	291	70.10	10.113 \pm .183	96.91	27.608 \pm .412
P	291	70.45	10.227 \pm .144	98.28	25.734 \pm .302
K	324	74.69	10.401 \pm .133	97.84	29.598 \pm .296
NP	297	77.44	9.783 \pm .158	96.30	25.428 \pm .410
NK	276	77.90	10.368 \pm .170	96.74	28.554 \pm .482
PK	297	73.06	8.952 \pm .098	97.31	26.280 \pm .258
NPK	285	77.54	10.515 \pm .159	95.79	27.990 \pm .383
Check	291	60.48	9.162 \pm .157	89.35	24.096 \pm .414

due to the application of fertilizers than would an active parasite such as *Penicillium expansum*. The omnivorousness of this fungus in nature, and its ability to grow readily on a wide variety of media (10, 29) lead one to expect only a slight effect on growth as a results of differences in the chemical composition of the fruit produced on trees in fertilizer and check plots. Certainly, the data show that modification of fertilizer practices alter internal resistance so slightly as to be unimportant in determining the incidence of blue-mold decay.

Another lot of the 1933 fruit, picked and stored in the same way, was coated with the tissue of decayed apples on December 17, 1933. On February 1, 1934, (46 days later) the fruit was examined and the number of lenticel infections by *Penicillium expansum* were recorded (Table 3).

TABLE 3.—Number of lenticel infections in Jonathan apples from various fertilizer plots resulting from coating fruit with decayed tissue. 1933

Fertilizer treatment	Number of apples	Percentage apples uninfected	Lenticel infections per apple	
			Mean	Maximum
N	40	2.50	7.375 \pm .586	23
P	35	2.86	6.914 \pm .631	21
K	42	9.52	5.714 \pm .458	16
NP	36	0.0	12.667 \pm .794	29
NK	32	6.25	7.188 \pm .613	20
PK	40	2.50	8.425 \pm .803	38
NPK	39	0.0	10.462 \pm .832	28
Check	39	5.13	7.462 \pm .476	24

No constant relationship was shown between the application of any of the 3 salts to the various plots and the resistance of the fruit to infection

at lenticels; these limited data suggest that fertilizer practices are unimportant in determining susceptibility to such infection.

EFFECT ON RESISTANCE OF MATURITY AT PICKING AND OF
LENGTH OF STORAGE PERIOD

This problem has been studied in England for several years during investigation of the relation of chemical composition to decay resistance and to the fungous succession during prolonged storage. The major attention has been given to slow-growing fungi appearing late in the storage season, but it has been realized (21) that *Penicillium expansum* is able to infect at any time in storage. Later work (19) showed that, of the 7 fungi studied *P. expansum* was surpassed only by *Phomopsis coneglanensis* Trav. in ability to infect at lenticels.

Brooks and Cooley (7) found that longer time was required for the development of measurable rot when *Penicillium expansum* was inoculated into green fruit than when placed in ripe apples at 0° C. Overholser and Latimer (25) found, in California, that late pickings of several varieties of pears generally were more susceptible to blue-mold decay than earlier pickings. The amount of decay in Australian and New Zealand apples (including Jonathan and Delicious varieties) at the time of unloading in England was found in the years 1927-1930 to be directly related to the fruit maturity (12). The authors (4) previously found a slight progressive increase of the number of blue-mold lenticel infections in Jonathan, Delicious, and Winesap apples in early-, prime-, and late-maturity pickings; the number was greatly increased in extra-late maturity Jonathans.

The effect of maturity of fruit and the length of storage period on the incidence of lenticel infection and the rate of advance of decay in the tissue was investigated in 1933. Delicious apples were picked from 2 trees at early maturity (October 2, 1933); in order to accentuate the difference between early and prime pickings only the most immature fruit was selected for the former. The prime-maturity picking was made on October 11, 1933, from the same 2 trees. Winesap apples were picked from 2 trees at early maturity (October 2, 1933), and prime maturity (October 16, 1933). All lots were packed without being washed, and shipped at once to Pullman.

Part of the fruit of each box was punctured and inoculated by the method described, and a nonpunctured portion was coated with the tissue of apples decayed by *Penicillium expansum*. The first inoculation for early-maturity Delicious was made on October 6, and the late-maturity picking was first inoculated on October 14. For the Winesap variety the first dates were October 6 and October 19, respectively. Four subsequent inoculations were made at 60-day intervals from these dates, the last being made in June. The studies thus covered a period exceeding that of ordinary storage practice.

The punctured portion of each box was examined and the size of the lesions determined 30 days and 60 days after inoculation. The nonpunctured fruit, coated with decayed tissue, was examined 45 days after inoculation, and the number of lenticel infections per apple was recorded.

The data on the mean diameter of decayed areas in Delicious and Winesap apples of early- and prime-maturity pickings inoculated at different times in storage are presented in tables 4 and 5.

It is apparent that the rate of decay in the second 30-day period was greater than in the first 30 days; in the Delicious series the advance in the second period was about twice that of the first; in the Winesap it was about $1\frac{1}{2}$ times that of the first period. This is in agreement with the conclusions of Brooks and Cooley (7) with the same organism.

TABLE 4.—Mean diameter of decayed areas in early- and prime-mature Delicious apples inoculated at various periods in the storage life of the fruit

Storage age of fruit (days)	Total punc- tures	Examined after 30 days		Examined after 60 days	
		Percentage punctures decayed	Mean diameter (mm.)	Percentage punctures decayed	Mean diameter (mm.)
Early-maturity picking					
0	261	96.17	13.140 ± .152	100.00	35.604 ± .242
60	240	78.75	11.520 ± .131	97.50	33.680 ± .353
120	249	26.10	10.754 ± .266	98.31 ^a	29.268 ± .392
180	261	65.13	10.953 ± .143	100.00	38.430 ± .271
240	129	80.62	16.590 ± .362	100.00	45.384 ± .499
Total or average	1140	68.33	12.591 ± .102	99.11 ^a	36.473 ± .162
Prime-maturity picking					
0	213	99.53	14.717 ± .236	99.53	41.310 ± .386
60	228	81.58	10.746 ± .150	99.56	34.224 ± .327
120	228	32.89	9.120 ± .177	99.56	33.304 ± .218
180	222	86.76 ^b	10.464 ± .151	100.00 ^b	39.168 ± .242
240	261	97.67 ^c	21.202 ± .188	100.00 ^d	51.475 ± .120
Total or average	1152	79.84 ^e	13.250 ± .082	99.73 ^f	39.896 ± .123

^a Exclusive of 4 apples infected by *Botrytis cinerea*.

^b Exclusive of 1 apple infected by *B. cinerea*.

^c Exclusive of 3 punctures in 3 apples infected by *B. cinerea*.

^d Exclusive of 7 apples infected by *B. cinerea*.

^e Exclusive of 6 punctures infected by *B. cinerea*.

^f Exclusive of 8 apples infected by *B. cinerea*.

The prime-maturity fruit consistently showed somewhat larger decayed areas than early-maturity pickings. The difference in the Delicious pickings

was $0.659 \pm .130$ mm. after 30 days, and $3.423 \pm .202$ mm. after 60 days. With the Winesap variety the difference was $1.201 \pm .110$ mm. after 30 days, and $3.518 \pm .200$ mm. after 60 days. Although these figures are statistically significant, the differences are rather small. In these series the percentage of punctures decayed showed that the prime-maturity fruit was slightly more susceptible to infection than that harvested earlier. It is felt that maturity at the time of picking is of small importance in determining internal resistance to the advance of *Penicillium expansum*, even though fruit picked early is slightly more resistant than that harvested at prime maturity.

TABLE 5.—Mean diameter of decayed areas in early- and prime-mature Winesap apples inoculated at various periods in the storage life of the fruit

Storage age of fruit (days)	Total punc- tures	Examined after 30 days		Examined after 60 days	
		Percentage punctures decayed	Mean diameter (mm.)	Percentage punctures decayed	Mean diameter (mm.)
Early-maturity picking					
0	360	58.33	8.736 ± .152	99.72	19.386 ± .252
60	348	25.29	10.293 ± .230	67.53	18.128 ± .402
120	342	28.36	8.784 ± .182	90.56 ^a	21.696 ± .347
180	339	32.45	9.543 ± .114	99.12	31.500 ± .237
240	312	72.76	13.344 ± .182	99.68	38.958 ± .202
Total or average	1701	43.03	10.140 ± .079	90.99 ^a	25.934 ± .133
Prime-maturity picking					
0	327	78.59	11.883 ± .151	100.00	27.991 ± .357
60	309	60.19	9.969 ± .177	90.29	25.806 ± .381
120	318	29.56	8.340 ± .178	86.48	23.682 ± .319
180	366	33.61	9.561 ± .122	96.16 ^b	28.764 ± .299
240	285	88.73 ^b	16.952 ± .200	98.92 ^c	41.018 ± .274
Total or average	1605	56.86 ^b	11.341 ± .075	94.36 ^d	29.452 ± .147

^a Exclusive of 1 apple infected by *Botrytis cinerea*.

^b Exclusive of 1 puncture infected by *B. cinerea*.

^c Exclusive of 7 punctures infected by *B. cinerea*.

^d Exclusive of 8 punctures infected by *B. cinerea*.

The effect of length of the storage period on the internal resistance of Delicious and Winesap apples to the advance of *Penicillium expansum* was variable in these investigations. Only small differences were noted during the first 120 days, but marked increases in the rate of advance occurred after 180 days, particularly with prime-maturity fruit. The ability

of this organism to grow readily on a wide range of substrata (10, 29) probably explains the fact that no consistent effect on growth rate occurred until late in the season. These results are not in disagreement with the findings of English investigators with less omnivorous and virulent pathogens. In general the internal resistance of apples to *P. expansum* may be said to be unimportant in determining the incidence of blue-mold decay in commercially stored fruit. As a single visible decay spot makes the apple a cull, a decrease in the rate of advance of decay is of commercial significance only in the early stages of invasion; the mere extension of the time required to produce complete decay is unimportant. The prolonged storage periods employed give ample time for the visible expression of infections of the wounds resulting from packing operations and of infections early in the season. Late-season lenticel infections and infections at wounds incurred in market handling become apparent rather rapidly, due to decreased internal resistance.

The effect of maturity of fruit and the length of the storage period on the incidence of lenticel infection in fruit coated with decayed tissue is shown in tables 6 and 7.

The prime-maturity picking showed more lenticel infections per apple than the early-maturity fruit in both the Delicious and Winesap varieties.

TABLE 6.—*Number of lenticel infections in early- and prime-mature Delicious apples coated with decayed tissue at various periods in the storage life of the fruit*

Storage age of fruit (days)	Number of apples	Percentage apples uninfected	Lenticel infections per apple	
			Mean	Maximum
Early-maturity picking				
0	36 ^a	0	7.000 ± .533	20
60	36	5.56	9.083 ± .757	34
120	37	2.70	17.459 ± 1.549	50
180	38	2.63	7.632 ± .625	26
240	31	6.45	11.742 ± .931	35
Total or average	178 ^a	3.47	10.583 ± .425	33
Prime-maturity picking				
0	26	0	30.654 ± 2.931	106
60	30	3.33	14.200 ± 1.060	37
120	32	0	13.375 ± 1.030	40
180	29	3.44	11.448 ± 1.140	33
240	33	0	18.182 ± 1.177	44
Total or average	150	1.35	17.572 ± .734	52

^a Including 3 apples each with one infection by *Botrytis cinerea* in addition to those by *Penicillium expansum*.

TABLE 7.—*Number of lenticel infections in early- and prime-mature Winesap apples coated with decayed tissue at various periods in the storage life of the fruit*

Storage age of fruit (days)	Number of apples	Percentage apples uninfected	Lenticel infections per apple	
			Mean	Maximum
Early-maturity picking				
0	50	24.00	2.740 ± .237	11
60	43	55.81	1.293 ± .079	4
120	53	43.00	1.849 ± .174	8
180	48	50.00	1.438 ± .103	5
240	43	4.65	6.721 ± .576	22
Total or average	237	35.57	2.808 ± .132	10
Prime-maturity picking				
0	46	2.17	6.348 ± .712	30
60	38	23.68	2.974 ± .339	16
120	44	29.55	3.523 ± .421	17
180	50	16.00	4.240 ± .366	17
240	39	0	6.615 ± .569	19
Total or average	217	14.28	4.740 ± .224	19.8

The difference in the case of the former was 6.989 ± 0.848 and in the latter was 1.932 ± 0.259 ; both of these figures are statistically significant. These differences are rather large in comparison with the average number of lenticel infections involved, being nearly half of the prime-maturity average in each case. The trend is the same as that obtained in 1931 (4) with Jonathan, Delicious, and Winesap apples inoculated with a spore suspension. From the standpoints of the maximum number of lenticel infections, the trend of increased susceptibility of the lot with increased picking maturity is also apparent. It is, therefore, concluded that the susceptibility of these varieties to lenticel infection (*i.e.*, the number of susceptible lenticels per apple) is increased materially by delayed harvesting.

There was shown in this study no consistent relation of the length of storage period and the susceptibility of the fruit to lenticel infections. Thus the number of infectible lenticels per apple did not change materially during storage. This is not surprising, since cutinization, the process involved in the development of resistant lenticels (9), does not proceed rapidly at low temperatures, and since the epidermal and subepidermal cells may die early in the storage season (2). It was found previously (4) that holding the fresh fruit at orchard temperature and humidity for 6 or 12 days or in dry air at 30° C. for 10 days tended to decrease the number of infectible lenticels per apple. Horne (19), working with *Penicillium expansum* and 6 other fungi, stated that "the results failed to show that age had any appreciable effect in modifying the external resistance."

VARIETAL SUSCEPTIBILITY

The varietal susceptibility of apples to infection at lenticels and to the radial advance of *Penicillium expansum* has received little attention.

Machacek (23) reported that the apple variety Russet was apparently "immune to outside infection." The destructive pinhole rot of pears on the Pacific Coast, a result of blue-mold lenticel infection, is much more prevalent in Winter Nelis than in any other variety (14, 27). Columbia, Doyenné d'Alençon, and Seckel pears have been reported (25) to be relatively immune from blue-mold decay, "apparently on account of the character of the tissues and epidermis." In tests with over 19,000 apples of the 1931 crop handled in various ways, the writers (4) found the decreasing order of susceptibility to infection at lenticels to be: Jonathan, Delicious, Winesap. Horne and Eweis (19) found that Worcester Pearmain apples were more susceptible than Bramley's Seedling to lenticel infection by *Penicillium expansum* and 4 other fungi.

Some work has been conducted to determine the susceptibility of different varieties of apples and pears to the radial advance of *Penicillium expansum* in the tissues. Machacek (23) found that blue-mold decay spread more rapidly in McIntosh than in Fameuse apples. Unpublished data⁵ of R. W. Welsh on the rate of advance of *P. expansum* in a limited number of apples of 12 varieties held at about 22° C. showed the decreasing order of resistance to be: York, Stayman Winesap, Winesap, Rome Beauty, Jonathan, Baldwin, Ben Davis, McIntosh, Wealthy, Delicious, Golden Delicious, and Grimes. The fruit was used at only one stage of maturity, and was inoculated on only one date, November 7, 1931.

The data presented in tables 4 and 5 consistently show that the rate of decay in the Delicious variety was more rapid than in the Winesap. In the early-maturity pickings the difference in mean diameter of the decayed area after 60 days was $10.539 \pm .210$ mm., and in the prime-maturity series it was $10.444 \pm .192$ mm. The same trend was shown by the percentage of punctures infected in each of these lots. The figures on the mean diameter of decayed areas were computed with the number of infected punctures taken as n and are, therefore, independent of the percentage of punctures that developed decay.

The data in tables 6 and 7 show that the Delicious was also consistently more susceptible to infection at lenticels than was the Winesap variety. The difference in the number of lenticel infections per apple in the early-maturity series was $7.775 \pm .445$, and in the prime-maturity fruit was $12.832 \pm .768$. The same relationship was displayed in 2 other ways; the Delicious showed a much lower percentage of noninfected fruit and higher maximum number of infections per apple than the Winesap.

⁵ Detailed data supplied by Dr. C. R. Orton, West Virginia Agricultural Experiment Station. Brief abstract in West Virginia Agr. Expt. Sta. Bull. 254: 52. 1932.

Varieties of apples differ greatly in the length of time they may be stored before they show senility and physiological breakdown. It has been shown that the susceptibility to lenticel infection by *Penicillium expansum* and the rate of advance of decay caused by it increase with delayed harvest and prolonged storage. A highly susceptible variety may be more resistant at the time of picking than a resistant one taken at the end of its storage life. It is apparent that any general study of varietal susceptibility to this organism must include tests on several stages of picking maturity made at a number of periods in the storage life. With varieties more dissimilar than those here employed, it is expected that a greater effect of time on internal resistance and the number of infectible lenticels would be shown. These points should be considered in studies of varietal susceptibility of apples to other decay fungi.

DISCUSSION AND CONCLUSIONS

In the limited data presented, no appreciable effect of the application of orchard fertilizers was shown on the incidence of susceptible lenticels, but decayed areas in apples from fertilizer plots consistently were somewhat larger than in fruit from check plots. Inasmuch as incidence rather than increment of rot is a criterion of condition in commercial handling, and as the number of lenticels and punctures infected is not greatly influenced by fertilizer applications, orchard fertilization is probably unimportant in determining the occurrence of blue-mold decay in the stored fruit. This is thought to be due to the ability of *Penicillium expansum* to grow readily on a wide range of substrata; less omnivorous fungi might be expected to show a greater sensitivity.

The percentage of punctures successfully infected, and the rate of spread of blue-mold in Delicious and Winesap apples picked at prime maturity was only slightly greater than in fruit picked at an earlier date. However, in these and earlier (4) investigations there was a significant increase in the mean number of infectible lenticels per apple as a result of delayed harvest. The same trend was shown by the percentages of apples without susceptible lenticels and by the maximum number per apple in each of the series. It is not known why the number of open lenticels per apple should increase with maturation on the tree, but decrease with holding of the picked fruit at orchard temperatures for a few days (4). The number of open lenticels per apple was shown previously to be very high in an extra-late maturity picking of Jonathan. It seems fairly clear that delayed harvesting is an important factor in determining the amount of blue-mold decay in certain lots of stored fruit. The increase in the number of open lenticels with the attendant increased chance of the apple becoming infected is of more commercial consequence than the increased rate of spread of decay in an apple that is already a cull.

The internal resistance of Delicious and Winesap apples varied little during storage until April (180 days), after which the rate of advance of blue-mold decay materially increased. The number of susceptible lenticels per apple sometimes showed this same increase, but to a lesser degree. It is thought that the slight effect of this factor was due to the good keeping quality of the varieties used; with early varieties, or varieties of short storage life, the effect would be greater after such periods of storage.

The English investigators have considered that a localized senescence in the region of the lenticel preceded infection of it by fungi, and that the advance of the weaker pathogens was then conditioned by the general senescence (*i.e.*, lowered internal resistance) of the tissue. This theory is best presented by Kidd (21). It is not yet possible to say definitely whether the senescent tissue-lenticel infection theory can be applied to *Penicillium expansum*. The evidence for such a relation is: (a) the sudden advance of incipient lenticel infections after removal of fruit from cold storage (4); (b) the increase in the number of susceptible lenticels per apple and the decrease of internal resistance with delayed harvesting or prolonged storage. On the other hand the omnivorousness of *P. expansum* and the lack of marked internal resistance to it in apples, the correlation of susceptibility and the noncutinized condition of lenticular cells (4, 9), the fact that reduction of the number of susceptible lenticels by exposure to warm dry air (4, 9) would be impossible if senescence were the factor involved, and the unimportance of the "vigor of development" of the fungus in the process of infection (4)—all indicate that localized senescence is unimportant in determining lenticular resistance to *P. expansum*.

Under the conditions of this and previous (4) tests the resistance, expressed either in terms of the rate of advance of decay or as the number of susceptible lenticels per apple, was greater in the Winesap than in the Delicious variety. The internal resistance was indicated by the mean diameter of lesions and by the number of punctures infected; the resistance to lenticel infection was expressed as the mean and maximum number per apple, and by the percentage of apples without susceptible lenticels.

The resistance of the lenticels to infection by *Penicillium expansum* is associated with cutinization of the cells forming the lenticel basin (9). It seems probable that the susceptibility of an apple to lenticel infection is a function of the number of open lenticels in it. Whether the internal resistance is due to the physical structure, the chemical composition, or both, is unknown; the English work with other fungi suggests that the second may be the main factor.

The evidence at hand indicates that the number of infectible lenticels per apple may be increased by delayed harvesting, by holding fruit in cold storage and subsequently washing it in hot solutions, by severely bruising the apples, and by prolonged storage.

Jonathan, Delicious, and Winesap, in the order named, show decreasing susceptibility to lenticel infection by *Penicillium expansum* and, in the case of the last 2, to its radial advance in the tissue. It is perhaps significant that this is also the increasing order of the length of storage life and of commercial importance of the varieties in Washington.

The practical application of these findings to the Washington apple industry is rather obvious. The application of orchard fertilizers, the varieties grown, and the length of time fruit is held in storage may well continue to be determined without consideration of their relation to incidence of blue-mold decay. However, the practice of leaving fruit on the tree past prime maturity should be discontinued unless decay has been found to be unimportant in the grower-lot in question. A detailed consideration of the methods for the prevention of blue-mold decay under Washington conditions has been presented in another place (5).

SUMMARY

The rate of advance of blue-mold decay in Delicious and Winesap apples under Washington conditions was found to be slightly greater in fruit from trees in fertilizer plots than from trees in check plots, and greater in fruit picked at prime maturity than at early maturity. The length of the storage period did not affect the rate of decay until after 180 days, when it materially increased.

The number of lenticel infections by *Penicillium expansum* per apple was greater in fruit picked at prime maturity than at early maturity. The incidence of such infections in apples was unaffected by the application of orchard fertilizers and by the length of the storage period up to 180 days, but after this period a slight increase sometimes was shown.

The Winesap variety was more resistant to lenticel infection by *Penicillium expansum* and to the radial advance of this fungus in the tissue than was the Delicious.

Fruit should be picked when mature in order to prevent decrease of its resistance to blue-mold decay. The other cultural and handling practices investigated should be determined by factors other than occurrence of this storage rot.

DEPARTMENT OF PLANT PATHOLOGY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON.

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LIBERATION OF NEUTRALIZED VIRUS AND ANTIBODY FROM ANTISERUM-VIRUS PRECIPITATES¹

K. STARR CHESTER

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It has been known for 10 years that, when normal rabbit blood serum is mixed with plant juice containing the virus of tobacco mosaic, the serum causes a reduction in the number of infections obtainable with the virus juice. If the serum of an animal that has been immunized with the virus is used in this way, the reduction in infectivity is much more marked (8, 10, 11, 12). The writer pointed out in 1934 (4) that both types of serum decrease the infectivity of the virus through a nonspecific inhibitory effect, and that immune serum, in contrast to normal serum, also has a specific neutralizing effect on the virus. A further study has been made on the nature of the neutralizing action of serum on virus, and the present paper reports the results of this study. Since the nonspecific inhibitory effect of normal serum constituents seriously interferes with the study of specific neutralization, it was found desirable to precede the work on neutralization by attempts to purify the serum so as to eliminate the nonspecific inhibitory factor as far as possible. The first section of this paper is, accordingly, devoted to experiments in antibody purification.

PURIFICATION OF VIRUS-IMMUNE SERUM

Numerous techniques have been used, up to the present, in the purification and concentration of antibodies. The techniques include desiccation, moderate heating, fractionation with ammonium sulphate, dialysis and electro-dialysis, precipitation by dilution, adsorption and elution, and treatment with copper chloride. An adequate presentation of this subject and introduction to the literature concerned has been given by Hartley (7).

The method finally adopted in the present case consisted of a moderate heating followed by ammonium sulphate precipitation and dialysis. Virus-immune rabbit serum was obtained in the customary manner and absorbed with healthy-tobacco juice to remove the precipitins for healthy-tobacco protein (5). The absorbed serum was heated at 57° C. for 2 hours. This process converts some of the water-soluble globulin into water-insoluble globulin, without affecting the antibody content. Protein was then precipitated by adding saturated ammonium sulphate solution until the ammonium sulphate

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concentration reached 30 per cent saturation. This precipitated protein fraction (euglobulin), which showed very little antibody activity, was filtered off and discarded. The filtrate was next precipitated by adding enough saturated ammonium sulphate to bring the ammonium sulphate concentration up to 43 per cent saturation. The resultant protein precipitate (pseudoglobulin) contained nearly all the antibodies of the serum, but only a small proportion of the total protein of the serum. The filtrate from this precipitation (albumin) contained much protein but was nearly or entirely free from antibodies. The pseudoglobulin precipitate was suspended in water or saline solution and dialyzed against water. During dialysis there was a heavy precipitation of water-insoluble globulin. This precipitation did not remove appreciable amounts of antibody. After dialysis the precipitate was removed by centrifuging, and to the supernatant fluid was added enough concentrated NaCl solution to bring the salt concentration to .85 per cent. Such purified serum was colorless, water-clear, and highly active, serologically.

The various protein fractions of normal serum were tested in a number of experiments to determine which fractions contained the substance or substances responsible for the nonspecific inhibitory property of serum. The results of the tests showed that this property is associated with the proteins, the dialyzable salt fraction being noninhibitory. Of the proteins, the albumin and euglobulin were each somewhat inhibitory to virus infection but less so than the pseudoglobulin. However, the removal of both the albumin and euglobulin fractions reduced the nonspecific inhibitory action of serum to a very considerable extent without affecting the antibody content.

As this method of purification of immune serum greatly reduces the protein content of the serum, it was thought that the precipitin titer of the purified serum might be unduly low, since it was possible that the nonantibody protein of the serum might contribute to the bulk of precipitate produced in the precipitin reaction. The nonantibody protein content of purified serum was, therefore, increased by adding various amounts of nontreated normal serum. No increase in precipitin titer resulted from this addition, indicating that in the purified serum there is sufficient protein to permit a maximum precipitin reaction.

Having developed a method for the purification of immune serum that removed a large proportion of the materials responsible for the host-inhibitory effect of the serum without removing the antibodies, it was feasible to proceed to an investigation of the nature of the neutralization reaction. The work was developed along two lines, namely, a study of the recovery of virus after neutralization with immune serum and a study of the recovery of antibody from serum-virus mixtures that contained no serologically demonstrable free antibody.

LIBERATION OF NEUTRALIZED VIRUS

Numerous methods for the purpose of recovering virus from serum-virus mixtures have been attempted in the past, but with relatively little success. Vaccinia virus is an exception. If this virus is treated with an adequate amount of serum from a recovered animal, the virus is neutralized so that a dose of the mixture administered to a normal animal fails to cause disease. If to a similar dose that would fail to cause infection are added several parts of saline diluent and the whole diluted mixture is inoculated into an animal, disease results (1). In this case the noninfective virus-antibody combination apparently has been dissociated simply by dilution. Reactivation by dilution also has been described for the viruses of fowl-plague, herpes, and poliomyelitis (2). Vaccinia virus and virus III of rabbits have both been recovered from neutral virus-serum mixtures by adsorption on kaolin or euglobulin (1). Techniques of washing, heating, and change in pH have all been utilized in attempts to separate virus and antibody. Weiss (14) reported the recovery of bacteriophage from a neutral bacteriophage-antiserum mixture through a digestion of the mixture with trypsin, but this was not confirmed by Muckenfuss (9). No other record of liberation of neutralized virus by enzyme action has come to hand.

In preliminary experiments, a number of techniques were used in attempts to recover tobacco-mosaic virus after neutralization. These techniques included dilution in saline solution or water, heating nearly to the thermal inactivation point of the virus, titrating with acid or alkali nearly to the extinction point of the virus, and treating with various chemicals, *e.g.*, .125 per cent AgNO_3 , .0625 per cent KMnO_4 , and .25 per cent chloramine-T. Virus antigen was not recovered when either serum-virus mixtures or the washed precipitate from such mixtures were treated in any of these manners.

It was thought that the immunization of a rabbit with serum immune from tobacco-mosaic virus might induce in the rabbit antibodies antagonistic to the action of the immune serum with which it was injected, *i.e.*, "anti-antibodies." This procedure was attempted. Rabbits were inoculated with 36 cc. of whole virus-immune serum, given in 6 biweekly intraperitoneal inoculations. The serum from the rabbits was mixed in various dosages with ordinary anti-mosaic serum and the mixtures tested for precipitating power against virus juice. It was found that the "anti-antiserum" had no demonstrable effect in inhibiting the action of the mosaic-immune serum. On the contrary, it behaved exactly like an ordinary anti-mosaic serum of rather low titer. The experiment shows that when mosaic-immune rabbit serum is inoculated into rabbits, it does not behave like a foreign body in inducing formation of antagonistic antibodies.

In addition to the experiments mentioned above, attempts were made to liberate neutralized antibody or virus by digestion of the serum-virus mix-

tures with pepsin. This procedure proved successful. The pepsin, under appropriate conditions, digested the antibody leaving the virus free and capable of acting both as a precipitinogen and as an infectious agent. Accordingly, the experiments with pepsin will be considered in some detail.

For recovery of virus precipitinogen, the following procedure was employed. Absorbed anti-virus serum was titrated against virus juice until neutralization was as complete as possible. In such neutralized mixtures virtually all of the virus and antibody were thrown down in the precipitate, leaving a supernatant in which precipitin tests showed the presence of no free virus or antibody. The more delicate infectivity test usually revealed some free virus in the supernatant fluids after neutralization. Even if a large excess of serum is added to virus, a few infections may be obtained from the mixtures, although relatively little serum is required to neutralize most of the virus. In this respect the neutralization of tobacco-mosaic virus by its specific antibody appears to resemble the neutralization of toxin by antitoxin. In the present study, mixtures of serum and virus were considered neutral when precipitin tests revealed no excess antibody or virus in the supernatant fluids of the mixtures. Infection tests of such mixtures showed a loss of most of the original infectivity of the virus used.

The mixtures or the washed precipitates from the mixtures after proper incubation were acidified and treated with crystalline pepsin, which was available through the courtesy of Dr. Roger M. Herriott. After incubation, the serum-virus-pepsin mixtures were titrated to pH 7 and centrifuged. The supernatant fluids then were found to contain large quantities of virus. This was detected either by precipitin tests or by infectivity tests. The following experiment is representative of several in which the precipitin method was used to detect liberated virus.

Absorbed anti-mosaic serum was purified according to the technique described in the previous section. To 1-cc. samples of the serum were added amounts of a concentrated virus juice varying from 2 cc. to .05 cc., with saline solution to adjust all the mixtures to the same volume. The mixtures were shaken and incubated for 2 hours at 37° C. and then overnight on ice. The following morning the amount of precipitate in each tube was noted. All of the tubes were centrifuged and the precipitates discarded. The supernatant fluids were each tested against undiluted absorbed anti-mosaic serum and against tobacco-mosaic juice at dilutions of 1:1 and 1:10. It was found that mixtures in which 1 cc. of serum had been added to more than .25 cc. of virus juice still contained excess virus, as shown by their reactions with the immune serum, while mixtures in which less than .25 cc. of virus juice had been added contained an excess of serum, as shown by precipitin tests. Accordingly, the mixture of 1 cc. serum with .25 cc. virus juice contained the optimum neutralizing proportions of serum and virus. A large amount of

serum-virus mixture was then made by adding 4 parts serum to 1 part virus juice. The mixture was incubated in the usual manner. The following morning a sample was centrifuged, tested, and found to contain an excess of neither serum nor virus. The mixture was then titrated to pH 3 and divided into 7 pairs of 1.5-cc. samples. To each sample was added concentrated pepsin and enough saline solution to adjust the volume to 3.5 cc. Each pair received a different amount of pepsin. One member of each pair was titrated back to pH 7.0 at once. This destroys the pepsin before it has time to act. The other member was titrated back to pH 7.0 after 5 hours' incubation at 37° C. The two members of each pair were treated identically throughout, except for the time when alkali was added. The 14 samples after incubation were dialyzed against saline solution for 16 hours and centrifuged. The supernatant fluids were then tested for precipitin reactions against tobacco-mosaic-immune serum and tobacco-mosaic-virus juice. The results of the tests are given in table 1.

TABLE 1.—*Recovery of neutralized virus by pepsin digestion, as shown by precipitin tests*

Sample	Pepsin ^a	Incuba- tion ^b	Precipitin test with					Residual ^c precipi- tate
			Virus-immune serum at:				Virus juice at:	
			1:1	1:2	1:4	1:8	1:10	
1	0.01	5 hrs.	t ^d	t	t	t	0	++
2	0.03	"	++	++	++	++	0	+
3	0.1	"	+++	+++	+++	+++	0	0
4	0.3	"	+++	+++	+++	+++	0	0
5	1.0	"	+++	+++	+++	+++	0	0
6	3.0	"	++	++	++	++	0	0
7	8.0	"	+	+	+	+	0	0
8	0.01	None	0	0	0	0	0	+++
9	0.03	"	0	0	0	0	0	+++
10	0.1	"	0	0	0	0	0	+++
11	0.3	"	0	0	0	0	0	+++
12	1.0	"	0	0	0	0	0	+++
13	3.0	"	0	0	0	0	0	+++
14	8.0	"	0	0	0	0	0	+++

^a Final concentration of crystalline pepsin in mg. of pepsin protein nitrogen per cc.

^b Period elapsing between addition of pepsin to acid mixture and titration to pH 7.0

^c Undigested precipitate from original precipitin reaction after pepsin treatment and titration to pH 7.0.

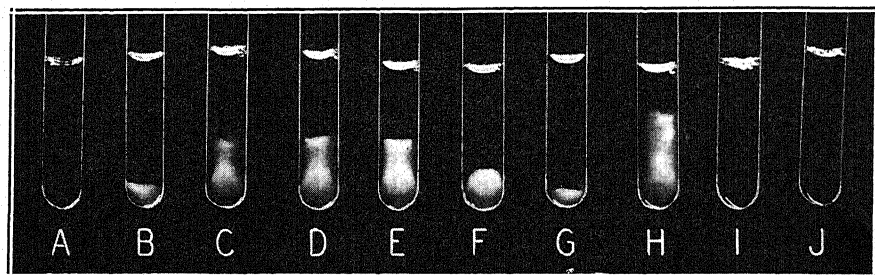
^d+++ = strong reaction.

++ = moderate reaction.

+ = reaction safely positive but not strong.

t = trace of reaction.

Consideration of the table brings out the following facts. Each of the mixtures on which pepsin was allowed to act for 5 hours subsequently yielded a supernatant fluid that contained virus precipitinogen, as shown by its reaction with mosaic-immune serum. In other words, virus that had been bound by the anti-virus antibodies was released by the digestion of the antibodies by pepsin. With .01 mg. protein nitrogen (P. N.) pepsin per cc., the release was small, and there still remained undigested precipitate; in the mixture receiving .03 mg. P. N. pepsin there was greater release of virus and less undigested precipitate. In the tubes receiving .1, .3, or 1.0 mg. P. N. pepsin, all of the precipitate was digested and a maximum amount of virus was released. In the tubes that received 3.0 and 8.0 mg. P. N. pepsin, all of the precipitate was digested, and at this high concentration of pepsin not only were the antibodies destroyed but part of the virus as well, as is shown by the decreasing precipitin reaction. In the control tubes receiving various amounts of pepsin, but in which the pepsin was not allowed time to act, there was no indication of digestion of the original precipitate, nor was any liberated virus demonstrable. Figure 1 is from a photograph of some of the tubes



Photographed by J. A. Carlile

FIG. 1. Recovery of neutralized virus by pepsin digestion, as determined by the precipitin method.

from this experiment. Tubes A-G show the precipitin reactions of the released virus in tubes 1-7 of table 1, respectively. Tube H shows the amount of precipitate that would have been obtained had all the virus been recovered. Tubes I and J are representative of the precipitin tests of mixtures that contained pepsin that was not allowed time to act. It will be seen that the yield of recovered virus is relatively high in tubes C, D, and E when these are compared with the yield in tube H.

The pepsin dosages for maximum virus recovery are rather high (.1 to 1.0 mg. P. N. pepsin per cc.) as compared with the amounts of pepsin usually required for protein digestion. This is in accordance with the known fact that antibodies in general are more highly resistant to enzymes (and other physical and chemical agencies) than are the other serum proteins. The antibodies for tobacco-mosaic virus appear to be more stable than the antibodies for certain other plant viruses.

It is concluded from these experiments that the digestion of a neutral mixture of tobacco-mosaic virus and its specific serum with pepsin liberates large amounts of virus, as shown by precipitin tests. It was felt that supplementary evidence of the liberation of virus should be obtained from experiments in which pepsin digestion of serum-virus mixtures was followed by infection tests. Such experiments were accordingly performed.

The serum-virus mixtures from experiments such as that described above were tested for infectivity to determine whether the recovery of virus antigen was correlated with a recovery of virus infectivity. It was soon learned that an adequate demonstration of the recovery of virus infectivity after pepsin digestion of a neutral serum-virus mixture required an entirely different scale of serum and virus concentrations from that required for the precipitin demonstration. The relatively high concentrations of serum and virus that are desirable in precipitin testing are unsuitable for infection tests (4), and, furthermore, mixtures of virus and serum containing no excess antibody or virus from the standpoint of precipitin testing often contain enough free virus to give numerous infections. After some exploratory work, a technique was designed that has regularly demonstrated the recovery of virus from serum-virus mixtures by means of pepsin digestion. The following representative experiment gives the essential features of the technique and results.

The antigenic solution used was a virus-containing tobacco juice that had been concentrated by two precipitations with half-saturated ammonium sulphate, and was about 10 times as infective as virus juice after freezing. This was diluted 1:1000 with saline solution (=1:100 dilution of crude

TABLE 2.—*Recovery of infectivity of neutralized virus as a result of peptic digestion*

Tube	Virus	Serum 1: 20	Mg. pepsin P. N./cc.	Hours incubated	Total no. lesions/total no. half- leaves	No. lesions/ half-leaf
1	1:100	Immune	1.0	4	11/17	0.65 ^a
2	"	"	"	0	3/17	0.18
3	"	"	0.1	4	16/18	0.90
4	"	"	"	0	7/18	0.40
5	"	"	0.01	4	17/19	0.90
6	"	"	"	0	16/19	0.85
7	"	Normal	1.0	4	111/20	5.60
8	"	"	"	0	163/20	8.20
9	"	"	0.1	4	72/16	4.50
10	"	"	"	0	119/16	7.50
11	"	"	0.01	4	182/18	10.00
12	"	"	"	0	199/18	11.00

^a These results calculated in table 3.

virus juice). To samples of the diluted virus were added equal parts of purified normal or virus-immune serum at 1:20 dilution. These mixtures of serum and virus (with saline solution + virus controls) were shaken and incubated for 2 hours at 37° C. and for 16 hours on ice. Each mixture was then titrated to pH 3 and pepsin was added. Control samples were titrated back to pH 7.0 at once, and test samples were allowed to incubate for 4 hours at 37° C. and were then titrated to pH 7.0. The samples were dialyzed for 16 hours against distilled water, and each sample was tested for infectivity by rubbing on 16–20 half-leaves of *Nicotiana langsdorffii* Weinm. The samples designated 1 and 2 in the tables to follow were rubbed on opposite halves of the same leaves, and similar tests were made with samples 3 and 4, 5 and 6, etc. The results of the experiment are summarized in tables 2 and 3.

It is apparent, when one considers the various substances and treatments involved in this experiment, that many factors contribute toward increasing or decreasing the number of lesions finally obtained. The neutralization of virus by immune serum decreases the lesion count. But the immune serum also contains normal serum proteins that participate in a nonspecific fashion in this decrease of infectivity (4). Besides destroying antibodies, pepsin also has effects on the normal serum inhibition and on the virus directly. The presence of denatured pepsin and pepsin digestion products in the mixtures, the action of the acid on the various constituents, and many other factors, all play a part in determining the number of lesions produced. In an attempt to obtain a somewhat accurate measure of virus recovery, the following method of calculation was employed.

In each test there were 4 observed values, i.e.:

- A. Number of lesions with virus + normal serum + denatured pepsin ;
- B. " " " " " + immune " + " " ;
- C. " " " " " + normal " + active " ;
- D. " " " " " + immune " + " " .

Value A is taken as representing the maximum amount of virus present and potentially infective in any of the 4 serum-virus mixtures (100 per cent). Neutralization without recovery through pepsin action removes the activity

of all but $\frac{B}{A}$. 100 per cent of the virus. The action of pepsin in the presence

of virus and normal serum decreases the infectivity of the virus by $\frac{C}{A}$, hence

$\frac{A}{C}$ is the necessary correction factor for the pepsin-treated immune-serum-virus mixture. The observed percentage of the original virus (A) present

in the immune serum-virus mixture after pepsin action is $\frac{D}{A}$. 100, and this,

when corrected for the pepsin-normal serum effect, becomes $\frac{D}{A} \cdot 100 \cdot \frac{A}{C}$. The actual percentage increase in infective virus due to the pepsin effect in liberating neutralized virus, then, is equal to the percentage of free virus after pepsin treatment minus the percentage of free virus before pepsin treatment, or $\left(\frac{D}{A} \cdot 100 \cdot \frac{A}{C}\right) - \left(\frac{B}{A} \cdot 100\right)$, which simplifies to $\left(\frac{D}{C} - \frac{B}{A}\right) \cdot 100$ per cent. This formula was used for calculating the percentages of virus recovery in tables 3 and 4. It is seen from table 3 that with virus at 1:100, immune

TABLE 3.—*Calculation of the data given in table 2*

	Mg. pepsin P. N./cc.		
	1.0	0.1	0.01
A (Lesions, virus + normal serum)	8.20	7.50	11.0
B (" " + immune ")	0.18	0.40	0.85
C (" " + normal serum + pepsin)	5.60	4.50	10.00
D (" " + immune " + ")	0.65	0.90	0.90
Recovery of virus $\left(\frac{D}{C} - \frac{B}{A}\right) \cdot 100\%$ ^a	9.4%	14.6%	1.3%

^a Per cent of neutralized virus in the mixture that was actually liberated through pepsin digestion of neutralizing antibody.

serum at 1:20, and 1 mg. P. N. pepsin acting for 4 hours, the amount of infective virus recovered in the mixture after pepsin action was 9.4 per cent of the maximum theoretical yield. With .01 mg. P. N. pepsin, the recovery of virus was less. It is apparent from the above table that pepsin action on normal serum increases the inhibiting effect of normal serum and, consequently, decreases the number of lesions produced. The pepsin effect on immune serum-virus mixtures includes this action to decrease the number of lesions opposed to the pepsin effect in releasing virus. Because the two effects act in opposite directions, it is necessary to remove the pepsin-normal serum action in order to observe the pepsin-immune serum action adequately. This is accomplished by the correction factor introduced into the formula. The smallest amount of pepsin used had little effect in increasing the nonspecific inhibitory effect of normal serum, while the larger amounts of pepsin increased this effect more markedly.

This type of experiment has been repeated a number of times, and in all cases, unless the amount of pepsin administered was relatively small, a marked recovery of infectivity was noted in the pepsin-immune serum-virus mixtures. Table 4 summarizes the results of these experiments.

TABLE 4.—*Recovery of neutralized virus by pepsin digestion as shown by infectivity tests. Summary of all experiments*

Test	Virus dilution	Serum dilution	Mg.P.N. pepsin per cc.	Pepsin incubation period	Observed number of lesions ^a				Per cent of total virus recovered ^a
					A	B	C	D	
1	1: 100	1: 20	1.0	4 hrs.	6.1	0.62	5.2	1.50	19.0%
2	"	"	"	"	8.2	0.18	5.6	0.65	9.4%
3	"	1: 10	0.2	2 hrs.	8.4	0.40	2.8	0.60	16.6%
4	"	"	"	"	28.0	1.30	6.0	2.00	28.6%
5	"	1: 20	0.1	4 hrs.	7.5	0.40	4.5	0.90	14.6%
6	"	1: 10	"	"	13.2	4.60	3.9	2.25	23.0%
7	"	"	0.08	5 hrs.	7.7	0.70	1.7	0.90	43.9%
8	"	"	"	"	27.0	0.20	11.0	2.10	18.3%
9	"	"	0.025	4 hrs.	4.0	0.00	5.0	0.20	(100 %)
10	"	"	"	"	16.0	0.20	3.0	0.90	28.7%
11	"	"	0.01	"	10.6	5.20	7.7	3.70	0 %
12	"	1: 20	"	"	11.0	0.85	10.0	0.90	1.3%
13	"	1: 100	"	5 hrs.	13.0	2.50	3.0	1.30	24.3%
14	"	"	"	"	56.0	8.10	11.0	8.00	58.0%
15	1: 1000	"	0.025	2 hrs.	1.8	0.50	1.4	0.40	0 %

^a For significance of symbols and method of calculating recovery of virus see table 3 and pertinent text.

From this table it is seen that recovery of virus occurred in 13 of 15 tests. In one of the two tests in which recovery was not observed (Test 11), the amount of pepsin was relatively small and the amount of serum to be digested was large, and in the other (Test 15), the virus was at relatively high dilution. On the whole, it appears that these infectivity tests support the precipitin tests in showing the recovery of virus from neutralized virus-serum mixtures. The absolute amounts recovered, as shown by the infectivity tests, are not so large as those recovered when tested by the precipitin method, probably because, under the conditions required for the infectivity tests, the virus is at relatively high dilution and, hence, more subject to pepsin digestion than concentrated virus (13).

It has been held by some serologists that antibodies possess their remarkable specificity because they are merely antigens that have been slightly modified by the serum proteins. If such a view be correct, one might anticipate that virus antigen could be recovered from virus antibodies by partial pepsin digestion of the antibodies. Tobacco-mosaic-immune serum is normally noninfective. Several experiments were performed in an endeavor to demonstrate virus infectivity in such serum after pepsin digestion, but in no case was evidence obtained that virus may be recovered from antibodies alone by pepsin digestion.

The present section has indicated that tobacco-mosaic virus, which has been neutralized by its specific immune serum, may be liberated when the antibodies are destroyed by pepsin digestion. The liberation of virus has been shown both by precipitin and by infectivity tests. This shows that when the virus is neutralized it is not destroyed, but is held in an impotent, noninfective condition from which it may be recovered.

At the time the foregoing studies on tobacco-mosaic virus neutralization were being concluded, a paper appeared by Bawden and Pirie (3) describing studies of the effect of pepsin on the "X" virus (latent mosaic or mottle virus) of potato. They found the "X" virus to be susceptible to pepsin digestion. Antibodies in general are relatively resistant to pepsin, and neutralized tobacco-mosaic virus was recoverable only because it was even more resistant to pepsin than the antibodies. The report that the "X" virus is susceptible to pepsin suggested that pepsin digestion of neutralized "X" virus might liberate antibody rather than virus. Accordingly, experiments were performed to test this possibility.

RECOVERY OF ANTIBODY FROM NEUTRAL ANTISERUM-VIRUS MIXTURES

Johnson's potato-ring-spot virus (a strain of the "X" virus) was employed in the following experiments. The virus was propagated in tobacco and the expressed juice of the tobacco plants was kept frozen until required. Immune serum was prepared in the usual manner, and the antibodies for healthy-tobacco protein were removed by precipitating the serum with 2 volumes of healthy-tobacco juice. Such absorbed serum was kept frozen until required.

In order to determine the optimal proportions of serum and virus for precipitation reaction, various amounts of virus-containing juice were added to serum. In performing the titrations the amount of precipitate formed in the mixtures was recorded, the tubes were centrifuged, and the supernatant fluids tested against antibody and virus to determine the presence, in the mixtures, of excess virus or antibody. The titrations showed that 4 volumes of juice were required to react with all of the antibodies in 1 volume of absorbed serum. Addition of more than 4 volumes of virus juice, however, did not result in the presence of excess virus until 30 or more volumes of virus had been added. In other words, 1 unit of antibody showed the property of reacting completely with 1, 2, 4, or 8 units of virus, but not more. This property of antibody is illustrated in the titrations reported in table 5.

From the table it is seen that with the given amount of virus, addition of more than $\frac{1}{4}$ part of serum resulted in an excess of antibody, while addition of less than $\frac{1}{32}$ part of serum resulted in an excess of virus, as shown by the precipitin tests. However, if the constant amount of virus was added

TABLE 5.—*Titration of "X" virus-containing juice with immune serum*

Tube	Undiluted virus juice (cc.)	Absorbed immune serum		Precipitate	Precipitin test of supernatant fluid with			
		Dilution	cc.		Virus 1:1	Virus 1:10	Serum 1:1	Serum 1:10
1	1	1:1	1	++ ^a	+++	+	0	0
2	1	1:2	1	++	+	+	0	0
3	1	1:4	1	++	0	0	0	0
4	1	1:8	1	++	0	0	0	0
5	1	1:16	1	++	0	0	0	0
6	1	1:32	1	+	0	0	0	0
7	1	1:64	1	+	0	0	+	+
8	1	1:128	1	0	0	0	++	++
9	1	1:256	1	0	0	0	++	++
10	1	1:512	1	0	0	0	++	++

Zone of neutrality

^a Symbols as in table 1.

to 1/4, 1/8, 1/16, or 1/32 parts of serum, reaction was complete and no excess antibody or virus was detected in the supernatant fluid.

The titrations having shown that 4 volumes of the virus juice would neutralize the antibodies in 1 volume of serum, a large quantity of serum was added to 4 parts of virus-containing juice. The mixture was incubated for 2 hours at 37° C.; the precipitate resulting was centrifuged, washed 3 or 4 times with distilled water, suspended in distilled water, and used in pepsin experiments.

Preliminary experiments showed that, when such precipitate was acidified and treated with pepsin, the virus was destroyed and antibody recovered. However, it soon became apparent that the pepsin was playing little or no part in this reaction, and that acidification alone sufficed to liberate antibody from the precipitates. A representative experiment will be described.

Washed serum-virus precipitate was divided into a series of samples. These were titrated to various pH levels by the addition of HCl. After incubating for 2 hours at 37° C., all of the samples were titrated to pH 7.0, the amounts of undissolved precipitate were noted, the tubes were centrifuged, and the supernatant fluids were tested against immune serum, normal serum, virus, and tobacco juice to detect liberated antibody or virus. The results of these tests are given in table 6.

TABLE 6.—*Recovery of antibody ("X" virus) by acidification of neutral serum-virus precipitate*

Tube	pH	Residual precipitate	Precipitin test of supernatant fluid with			
			Virus 1:1	Immune serum 1:1	Healthy tobacco 1:1	Normal serum 1:1
1	7.0	++a	0	0	0	0
2	6.0	++	0	0	0	0
3	5.5	++	0	0	0	0
4	4.8	(+) ^b	++	0	0	0
5	4.0	(+)	++	0	0	0
6	3.0	(+)	++	0	0	0
7	2.5	(+)	++	0	0	0
8	2.0	(+)	++	0	0	0
9	Untreated immune serum ^c		++	0	0	0
10	Untreated virus ^c		0	++	0	0
11	Original supernatant fluid		0	0	0	0
12	Wash fluid 1		0	0	0	0
13	Wash fluid 2		0	0	0	0
14	Wash fluid 3		0	0	0	0
15	Wash fluid 4		0	0	0	0

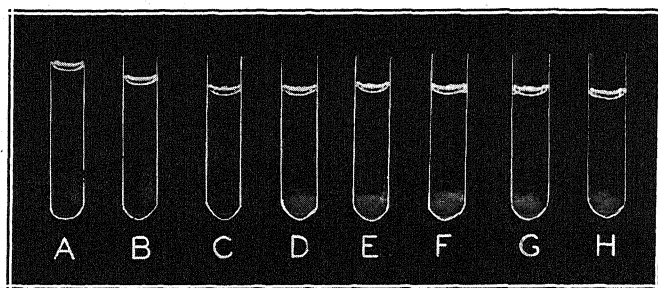
^a Symbols as in table 1.

^b Precipitate present but not of the type produced by precipitin reaction. Evidently a precipitate of inactive virus residue.

^c Adjusted to a concentration equal to that of serum or virus in original precipitate suspension (= maximum possible yield).

From the table it is seen that the precipitate could be taken to pH 5.5 without dissolution of the precipitate or liberation of its constituents. Titration to pH 4.8 to 2.0 resulted in dissolution of the neutral precipitate and the liberation of antibody. The yield of antibody was virtually 100 per cent, as far as could be told by the amount of precipitate when tested against virus (compare tubes 4–8 with tube 9). The accompanying photograph illustrates certain of the tubes of this experiment (Fig. 2). In the photograph, tubes A–C were of the mixtures incubated at pH 7.0, 6.0, and 5.5, respectively, tubes D–G were of the mixtures at pH 4.8, 4.0, 3.0, and 2.0, respectively, and tube H is the control tube (9 in the table), showing the amount of precipitate to be expected, had all of the antibody been recovered.

From experiments of this type, it is concluded that the acidification of neutral "X"-virus-antiserum precipitate at pH 4.8 to 2.0 results in a dissolution of the precipitate and a liberation of large quantities of antibody.



Photographed by J. A. Carlile

FIG. 2. Recovery of "X"-virus antibody by acidification of neutral virus-antibody precipitate.

DISCUSSION

It has been seen in the foregoing pages that the digestion of precipitates of tobacco mosaic and its specific immune serum liberates large quantities of virus, and that the acidification of precipitates of "X" virus and its specific serum liberates large quantities of antibody. From these results it is apparent that in the cases mentioned neutralization of virus by serum does not involve the destruction of either virus or antibody, respectively.

These findings provide serological methods for the purification of either virus or antibody. Such methods differ from the chemical methods usually employed, in that they are much more narrowly specific than chemical techniques for protein fractionation. The methods are not complicated and the yields of virus or antibody are high, of the order of 75 per cent to 100 per cent. It would be very desirable to utilize such methods in the preparation of purified antibody and virus, and then to compare the products obtained with those that have been obtained by the ordinary chemical methods.

Titration with the "X" virus indicate a considerable versatility of the antibody molecule, since it may combine with and be saturated by any number of units of antibody from 1 to 8. This is in harmony with Bordet's colloidal adsorption theory of toxin-antitoxin neutralization.

SUMMARY

Virus-immune serum, from which the antibodies for healthy-tobacco proteins had been removed, was purified by eliminating from the serum the fraction of protein that was insoluble in 30 per cent saturated ammonium sulphate and that soluble in 43 per cent. The pseudoglobulins rendered insoluble in water by heating to 57° C. also were eliminated. The resulting water-clear fraction after dialysis had suffered little or no loss in virus-antibody content, but showed only a fraction of the nonspecific inhibitory action of unpurified serum. The inhibitory property of normal serum was found to be distributed among all of the protein fractions of the serum.

Neutralized mixtures of tobacco-mosaic virus juice and immune serum were prepared by titrating the serum with the juice until the supernatant fluid after centrifuging contained an excess of neither serum nor virus, as determined by precipitin testing. When such mixtures were subjected to a number of chemical, physical, and serological treatments, no free virus nor antibody was recovered, but when the mixtures were partially digested with pepsin, the antibodies were destroyed and a large portion of the virus was recovered, as determined by precipitin tests.

Using a somewhat different experimental arrangement, for reasons that are explained, it was possible to show that the partial digestion of mixtures of immune serum and virus with pepsin increased the infectivity of the mixtures through the destruction of antibody and consequent liberation of virus. No infectious matter was obtained by the partial digestion of virus-free immune serum by pepsin, which provides evidence that the virus antibodies are not merely virus particles that have been modified by the serum proteins.

These findings demonstrate that, when tobacco-mosaic virus is neutralized by its specific immune serum, the virus is not destroyed, but is held in an impotent, noninfective condition from which it may be liberated if the antibodies are destroyed by pepsin digestion.

When neutral precipitates of potato "X" virus and its specific serum were acidified to pH 4.8 or below, the precipitate underwent dissolution and large amounts of free antibody were recoverable in the supernatant fluids. This indicates that, in the neutralization of this virus by its specific serum, the antibody is not destroyed.

Titration of "X" virus with its immune serum showed that 1 unit of antibody has the power of combining with and being saturated by any number of units of antigen from 1 to 8.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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THE RELATIONSHIP OF CEPHALOSPORIUM ACREMONIUM TO THE BLACK-BUNDLE DISEASE OF CORN

M. R. HARRIS¹

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INTRODUCTION

In fields of corn there frequently are plants that have made an abnormal growth. These abnormalities include barren stalks, nubbin ears, multiple ears at one node, excessive suckering, dwarfing and a purpling or reddening of the leaves. When such abnormal stalks of corn are split open and examined, a part of them will show discolored vascular bundles ranging from light brown to black. (Fig. 1, A). This abnormal condition has been found also in stalks having a normal external appearance and is known as the black-bundle disease of corn. It has been reported from all parts of the United States wherever corn is grown. Recently it has been reported from Kenya Colony, Africa (8). The black-bundle condition has been said to be due to various causes such as genetic factors, (9) environmental factors, and the attacks of fungi, but is most commonly believed to be associated with an invasion of the vascular bundles by the fungus *Cephalosporium acremonium* Corda.

REVIEW OF THE LITERATURE

Symptoms associated with the black-bundle disease of corn have been noted by practical corn breeders in Illinois since 1901. In 1916, Pammel, King, and Seal (6) reported that barren stalks, nubbins, and slow growing stalks were due to an invasion of *Gibberella saubinetii*. In 1920, Norton and Chen (5) studied the black-bundle condition in sweet corn and reported it to be due to an invasion of *Cephalosporium sacchari* Butler and Kahn, which, they then believed, were a *Fusarium* stage to develop, probably would have to be referred to *Fusarium moniliforme* Sheld. Manns and Adams, (4) in 1921, found *C. sacchari* in kernels of sweet corn and referred to it as a parasitic fungus that could be detected when corn was sprouted on the germinator. Manns, (3) in the same year, reported 39.54 per cent of all the seed corn in Delaware was infected by *C. sacchari*. Black bundles in the nodal area were said by Hoffer and Carr (1) to be due to phosphorus deficiency in the soil, which resulted in an accumulation of iron and aluminum salts in the nodal plate and the bundles passing through it.

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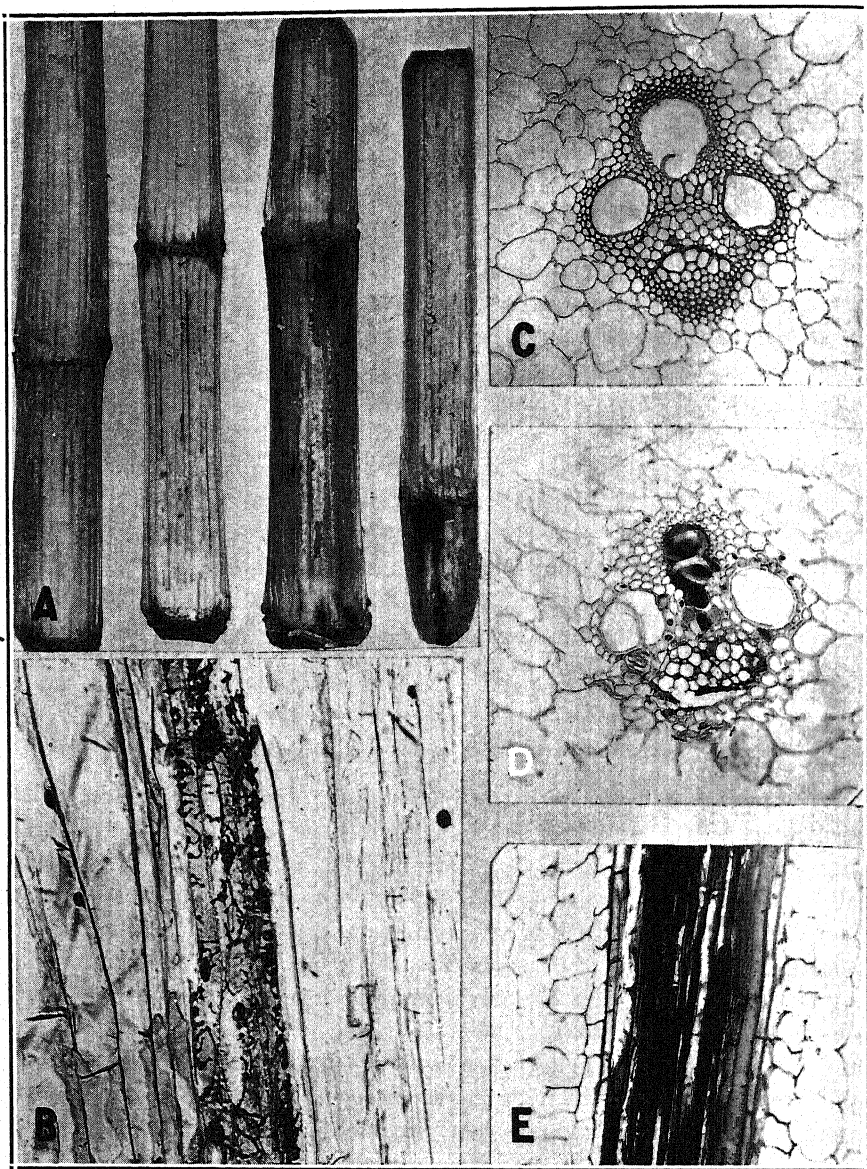


FIG. 1. A. Longitudinal sections through cornstalks showing discolored vascular bundles. B. Longitudinal section of a seedling corn-plant root invaded through an injured end by *Cephalosporium acremonium*. C. Cross-section of a normal vascular bundle of corn. D. Cross-section of a vascular bundle filled with gum. E. Longitudinal section of a vascular bundle filled with gum.

The most extensive study of the black-bundle disease of corn was published by Reddy and Holbert (7), in 1924. They found that the blackened vascular bundles, in plate cultures, very often produced *Cephalosporium acremonium*, which was mistaken by Manns and Adams for *C. sacchari*. Reddy and Holbert reported that the blackened condition of the bundles was due to an invasion of that fungus. They associated many corn abnormalities, such as multiple ears at one node and excessive sucker production, with the presence of black bundles. They reported that the fungus was seed-borne and completed its life cycle within the host. They stated that they demonstrated definitely the pathogenicity of the organism by injecting a spore suspension into the plant by means of a hypodermic needle. In 1930, Koehler and Holbert (2) in an extensive bulletin on the corn diseases of Illinois, listed the black-bundle disease. They stated that the black-bundle condition usually is caused by an invasion of *C. acremonium*, but that there is a possibility of its being caused by other factors. In 1935, Thorold reported from Kenya Colony, Africa, that the black-bundle disease was found there. He stated that *C. acremonium* was not always associated with the black-bundle condition (8).

METHODS OF EXPERIMENTATION AND DATA OBTAINED

In order to determine fully the relationship between the black-bundle condition and the fungus *Cephalosporium acremonium*, two methods of experimentation were used. First, corn plants showing the presence of black bundles in the field under natural conditions were examined, plated, and portions of the black bundles were fixed in formol acetic alcohol. Later, pieces of this fixed material were imbedded in paraffin, sectioned, and stained for microscopic study. Second, corn plants both in the field and in the greenhouse, were inoculated with cultures of the fungus in an effort to produce blackened bundles and abnormal growth characters. Along with this work, the effect of unfavorable environmental factors was noted as an influence in the production of black bundles.

THE BLACK-BUNDLE CONDITION

From January, 1928, to June, 1933, a study was made of the occurrence of the black-bundle condition. Experiments were carried on in the winter in the greenhouse and in the summer in the field. Several inbred strains of corn were used that have shown black bundles when grown under varying field conditions. These strains were developed by J. R. Holbert, Bloomington, Illinois, and have been inbred several years. One of these strains, known by the symbol A1237, has been under observation in this study for 5 years at Madison, Wisconsin; Bloomington, Illinois; and Sacramento, California. In 1928, this strain was grown at Madison, along with 26 other inbred strains,

and when examined at the end of the season, was found to have a large proportion of dark to black vascular bundles. This condition in the A1237 strain has been observed in every stalk examined during this study.

In general, the bundles were not a dense black but varied from light to deep brown. When a stalk was cut in cross section at the center of the internode, the darkened bundles usually were few in number. More were found in the lower than in the upper internodes. When viewed in longitudinal section, these bundles were found to vary in color in the same internode. Usually, they were darkest near the node and grew progressively lighter toward the center of the internode. Sharp variations in intensity of color frequently were noted in the same bundle. For one or two centimeters it would be very dark and abruptly would show scarcely any coloration. Actual counts showed that the number of darkened bundles per internode varied considerably. It was found that they were more numerous in the lower than in the upper internodes. In some of the stalks it was found that the darkened bundles were fewer in the lower than in the higher internodes. Strain A1237 was observed as having a darker discoloration of the bundles when grown on phosphorus-deficient soils showing excess nitrogen. On such soils the strain exhibited such abnormalities as decreased vigor of stalk growth, flamed and colored leaves, and no ears. This condition has been general in this strain grown under these conditions. The consistent appearance of the black bundles in this strain, regardless of where it is grown, suggested that the black-bundle condition was associated with hereditary tendencies.

Other strains of corn have been observed to have blackened bundles at times. Under the severe drouth conditions of 1930, in central Illinois, four other inbred strains of corn were found that, when examined, showed the black-bundle condition. These strains are known by the symbols BR10, AR9, G, and Illinois Low Ear. During years of normal soil moisture, these strains rarely showed black bundles, and have not shown the black-bundle condition when grown on soils deficient in phosphorus.

RESULTS OF PLATING OF BLACK BUNDLES

When it had been definitely demonstrated that black bundles occur regularly in the A1237 strain of corn, correlation of the presence of the fungus *Cephalosporium acremonium* with this condition was attempted, as has been reported by Reddy and Holbert (7). A series of platings was begun when the plants were about a foot high and completed after the stalks were mature. Black bundles were found in the stalks during this entire period. Internodes that were to be plated were cut from the stalks and freed of leaves and sheaths. They were washed in bichloride of mercury, split lengthwise with a sterilized scalpel, and a portion of the blackened bundles removed aseptically to a sterile test tube containing potato-dextrose agar.

The majority of the black bundles plated did not contain active cultures of *C. acremonium*, for less than 4 per cent of the cultures yielded the fungus. (Table 1.) Later, additional platings from the A1237 strain were made. Out of a total of 174 plants examined, slightly more than 4 per cent produced cultures on agar plates. When averaged in with the data presented in table 1, exactly 4 per cent of 343 stalks of the A1237 strain produced cultures of *C. acremonium*.

TABLE 1.—Summary of platings of roots and black bundles of corn from June 21, 1929, to October 8, 1929¹

Date of platings	Part of the plant	Cultures from platings			
		<i>Cephalosporium acremonium</i>	Other fungi	Free of fungi	Totals
June 21, 1929	Roots	0	0	10	10
27, 1929	"	0	0	10	10
July, 9, 1929	Internodes	0	0	10	10
16, 1929	"	0	0	10	10
23, 1929	"	3	0	17	20
Aug. 2, 1929	"	0	0	5	5
11, 1929	"	0	0	4	4
18, 1929	"	0	0	20	20
29, 1929	"	0	0	20	20
Sept. 19, 1929	"	3	8	9	20
24, 1929	"	0	4	16	20
Oct. 8, 1929	"	0	0	20	20
Totals		6	12	151	169
Per cent		3.8	7.5	88.7	100

¹ Platings made at Madison, Wisconsin.

Black bundles from strains other than A1237 were plated and found to contain an occasional trace of the fungus. Platings were made from the inbred strains BR10, AR9, G, and Illinois Low Ear, in 1930, when these strains developed blackened bundles. Out of a total of 77 stalks examined, less than 4 per cent were infested with the fungus. In summarizing the data of all the platings of black bundles it was found that 3.9 per cent of the 420 stalks of inbred corn were infested with active *Cephalosporium acremonium*. Nine and five-tenths per cent showed cultures of a number of other fungi, the one occurring most often being *Diplodia zeae* (Schw.) Lev. Of all the stalks examined, 86.6 per cent were fungus-free.

HISTOLOGICAL STUDIES

Darkened vascular bundles from which the fungus was plated, together with sterile bundles, were fixed, imbedded, sectioned, and stained with Dela-

field's haematoxylin and counter-stained with orange G in clove oil. The fungus mycelium stained a deep purple. Sections of bundles, known to be free of the fungus, showed a gum-like substance deposited in the cells (Fig. 1, D-E), which remained unstained. This substance ranged from light yellow to very dark brown. In bundles showing light deposits, the gum-like substance was located in the sieve tubes and companion cells of the phloem. In the more heavily impregnated bundles, the xylem vessels were often plugged and, in extreme cases, all of the cells of the bundle were filled with gum. In sections where the fungus was present, the mycelium was confined almost entirely to the xylem vessels and closely associated with the gum. Occasionally, hyphae were observed to pass through vessel pits into other cells of the bundle. Frequently, the fungus formed a mat of mycelium on one side of a vessel wall, but was never observed completely plugging a vessel. In plants grown under field conditions the fungus always was found associated with bundles containing some of the gum-like substance.

MICROCHEMICAL STUDIES

Microchemical studies on fresh and fixed materials showed the gum to be of a complex chemical nature. The material was fixed in formol-acetic alcohol and then dehydrated. It was then imbedded in paraffin, sectioned, cleared in xylol, and placed in absolute alcohol. It was then ready for the microchemical test as outlined by Eckerson. For fixed tissue the test is as follows:²

Put sections on a slide in one per cent. phloroglucin solution. (0.1 gram phloroglucin; 10.0 c.c. alcohol); add a drop of concentrated hydrochloric acid and observe. Lignin immediately turns violet. Heat carefully for about ten minutes. Pentoses become cherry red.

The pale yellow gum-like substance gave a cherry red reaction. The darker portions of the gum were not specific in their color reaction.

Fresh material was tested for pentoses with orcin. Freehand sections, cut from the internodes of newly harvested corn stalks, were kept in sterile distilled water until tested. The fresh sections were placed in a 4 per cent orcin solution. The excess solution was drained off with filter paper, and a drop of concentrated hydrochloric acid added. According to Eckerson,² lignin becomes immediately violet, without heating; the pentoses a blue to violet, after heating. The lighter colored gum deposits sometimes showed the blue or violet color, and at other times remained the original yellow, indicating considerable variation in composition. The darker portions of the gum always remained a deep brown and were not in any way acted upon by the chemicals used. In both fixed and fresh materials these tests would

² Eckerson, Sofia H. Microchemistry. [Unpublished mimeographed manuscript of the Department of Botany, University of Chicago.]

indicate that substances of a pentose nature were sometimes associated with the gum-like deposits.

Varied tests were run to determine the relative solubility of the mass of gum. When freshly cut freehand sections were put into 0.5 per cent solution of ammonium oxylate, and the solution heated to the boiling point, a noticeable dissolving of the lighter colored gum occurred. When these same sections were treated further with a 4 per cent solution of sulphuric acid, slightly more of the lighter portions of the gum were dissolved out. The residual material, and especially the darker colored mass of the deposit, was not hydrolysed when heated in strong acids at high temperatures.

FACTORS FAVORING GUM PRODUCTION

During the progress of the field study it was observed that corn plants of strain A1237, when grown in soil low in available phosphorus and having an excess of nitrogen, always contained more of the black bundles than were found in plants of this strain grown in a balanced fertile soil. In contrast, the BR10 strain did not react the same on the phosphorus deficient soil; but during the drouth in 1930, it did show black bundles. The results were checked in greenhouse experiments. The two strains of corn were grown in white sand in culture cans to which a balanced and an unbalanced nutrient solution were added by substituting KNO_3 for KH_2PO_4 in the control.³ Culture cans with normal soil, deficient in moisture, also were used.

Both strains of corn behaved under greenhouse conditions as they did in the field. The results (Table 2), show that black bundles were produced in strain BR10 when it was grown under drouth conditions but not when grown under conditions of unbalanced nutriment. Just the opposite effect was obtained with strain A1237, which produced fewer black bundles than usual on dry soil, but did produce numerous black bundles on an unbalanced nutrient solution. These results indicate that the presence of black bundles in the inbred lines of corn studied may be produced by different environmental changes, with a specific reaction in different inbred lines.

TABLE 2.—*Growth of two strains of corn under varying nutritional and moisture conditions with reference to the presence of the black-bundle condition*

Conditions of growth	Strain of corn	
	A1237 bundles	BR10 bundles
In moist soil	Lightly discolored	No discoloration
In dry soil	Very lightly discolored	Heavily discolored
Balanced nutrient solution	Lightly discolored	No discoloration
Unbalanced nutrient solution	Heavily discolored	No discoloration

³ Detmer's nutrient solution: Water, 1000 g.; $\text{Ca}(\text{NO}_3)_2$, 1.00 g.; KCl , 0.25 g.; MgSO_4 , 0.25 g.; KH_2PO_4 , 0.25 g.; FeCl_3 , trace.

THE ORGANISM FOUND ASSOCIATED WITH BLACK BUNDLES

The fungus isolated from the small percentage of the black bundles and classified as *Cephalosporium acremonium* corresponds with that described by Reddy and Holbert (7) as the cause of the disease. The colonies of the fungus are orbicular, dense, floccose, with the mycelium at first very thin, hyaline, branched, and sparsely septate. The conidiophores are erect, simple, and arise laterally from vegetative hyphae. They are uniseptate and 40 μ to 60 μ high. The conidia are numerous, elliptical or oblong, straight or curved, and nearly hyaline. They are very small ($4.0 \times 1.5 \mu$) and are borne singly at the apex of conidiophores. Each spore is pressed to one side by the next spore produced and they all adhere, by means of a slime, forming a head. The spore heads easily fall apart when submerged in water and the spores are freed.

When grown on different media, colonies of the fungus vary greatly in macroscopic characters. Some colonies are resupinate and creep along the surface of the medium. Others have a dense, felt-like mycelium, or coremium-like structures. Some colonies tend to assume a spiral growth to the right or left. Cultures do not remain constant in their characters, but over a period of time through several transfers they may show all the described variations of macroscopic appearance. At the lower temperatures, the fungus is white, but at higher temperatures it may become a delicate rose pink. It makes its best growth at relatively high temperatures. Petri-dish cultures were incubated for 2 weeks at temperatures ranging from 16° to 36° C., and the rate of growth was determined by the diameter of the colonies. It was found that the optimum temperature for mycelial growth was about 30° C.

The fungus grows well on any medium containing an available source of carbon. It makes a vigorous growth on potato-water agar containing a 6-carbon sugar, such as dextrose, lactose, galactose, maltose, and saccharose. It makes a similar growth when dextrin is the source of carbon. On medium containing the 5-carbon sugar xylose, the fungus made a growth similar to that on any of the hexose sugars. The growth on xylan, isolated from corn cobs, was twice that made in the same time on the hexose sugars. Growth was vigorous on corn meal-dextrose agar, and oatmeal agar media. A lesser growth was made on nutrient agar, bile agar, and agar containing Richard's solution. The fungus developed little aerial hyphae on these last 3 media, the cultures having much the same appearance as cultures of bacteria.

INOCULATION STUDIES IN THE GREENHOUSE

Several methods of inoculation with the organism were used on young and older corn plants grown in the greenhouse. After the roots of seedlings

had been injured, to give the fungus a possible means of entry, the seedlings were placed in soil containing the organism. Older plants were inoculated by pouring a spore suspension over the uninjured roots, and over the roots injured in various ways (Fig. 2). The most extensive type of inoculation



FIG. 2. A. Two plants of strain A1237 inoculated by pouring spore suspension of *Cephalosporium acremonium* over severely injured roots. B. Two plants of strain L111 inoculated as in A.

was carried on with a hypodermic needle. The fungus was cultured on agar and washed to obtain a spore suspension. This suspension was then hypodermically injected into the stalk of the young plant. In addition to these

methods of inoculation, plants grown from naturally infected seed were observed for disease symptoms and cultured to determine the possible presence of the fungus.

Corn seedlings were inoculated with the fungus through root injuries and then carried to maturity. Kernels of strain A1237 were surface-sterilized and then grown on agar. Those kernels, free of fungi and bacteria, when the seedlings had reached the four-leaf stage of development, were transferred to large test tubes containing sterile soil. The young plants continued to develop normally in these tubes for 2 weeks. They were then removed from the tubes and the ends of the roots were injured with a scalpel. They were transplanted to sterilized soil that had been inoculated with a spore suspension of *Cephalosporium acremonium* (Fig. 1, B). After ten days in this soil, several injured root ends from each plant were plated. The plants were carried to maturity. All of the root ends plated produced the fungus; but out of 20 of these plants, which were carried to maturity, no cultures of the fungus were plated from the vascular bundles.

Corn plants growing in soil to which a heavy spore suspension had been added at varying intervals during the growth of the plant reached maturity without infection. Corn plants having darkened bundles, such as strain A1237, became infected when the roots were injured at the time the plants reached the silking stage. No infection resulted from wounded roots being exposed to the fungus prior to silking. Plants that did not show the black-bundle condition were not invaded by the fungus through root injuries at any time during the life of the plant. The data on this series of root injury inoculations are given in table 3.

TABLE 3.—Summary of data on root inoculation experiments in the greenhouse

Stage of growth when inoculated	Strain	Cultures from platings			
		<i>Cephalosporium acremonium</i>	Other fungi	Free of fungi	Totals
6th leaf stage	A1237	0	22	108	130
Tassels showing	A1237	0	0	75	75
Tassels showing	BR10	0	0	75	75
Silk showing	A1237	20	0	0	20
Silk showing	BR10	0	0	10	10
Silk showing	L111	0	0	10	10

There appeared to be no difference in the relative pathogenicity of 6 isolations of the fungus. Macroscopically, these cultures varied considerably. Two of the isolations were made at Bloomington, Illinois, 2 at Massillon, Ohio, and 2 at Madison, Wisconsin. Spore suspensions of the 6 isolations were

inoculated into plants when 2 feet high by means of a hypodermic needle. The strain BR10 was inoculated. The fungus was recovered from every stalk inoculated. Externally, the plants appeared to be normal, and the fungus progressed but a very short distance from the point of inoculation.

Reddy and Holbert (7) reported that infected ears carry the organism internally in the seed; that the fungus develops with the germinating kernel and causes a systemic infection of the plant through the vascular system; and by this means, invades the ears and eventually the kernels. They believed that in this manner the fungus is carried over to the following season. A study of this phase of their work failed to bear out their findings.

Seed infected with *Cephalosporium acremonium*, but free of all other fungi and bacteria, was secured by plating from infected ears. Kernels were surface-sterilized, plated on potato dextrose agar, and only those with a pure culture of the fungus growing out of them were used. The seedlings were planted in sterilized soil and grown to maturity. Three out of 30 such stalks, when plated at maturity, yielded cultures of the fungus from the bundles. It was not possible to determine whether these 3 stalks were infected when the kernels germinated or whether infection took place some time later during their growth. All stalks were of strain A1237 and contained dark bundles.

FIELD INOCULATIONS

In order to determine the effect of an invasion of the fungus on field-grown corn plants, inoculations were made with a hypodermic needle. At Madison, Wisconsin, 3 strains of corn, A1237, BR10, and L111, were inoculated hypodermically. One hundred plants of each strain, approximately a foot high, were inoculated with a spore suspension. One hundred check plants of each strain were given a hypodermic injection of distilled water. Two hundred plants of each strain, in the silking stage, were given a hypodermic injection of the spore suspension. An equal number of uninjured plants of each strain, growing adjacent to the inoculated strains, served as controls. When examined in the field under conditions of normal growth and without inoculation with the fungus, strain A1237 always showed black bundles; strain BR10 showed black bundles occasionally, and strain L111 rarely, if ever, showed the black-bundle condition.

Externally, strain A1237 alone showed any effects of inoculation. A very small proportion of these stalks, inoculated when a foot high, appeared to be stunted in the same way as those described by Reddy and Holbert (7). However, as much stunting appeared among the controls injected with sterile distilled water. In other strains, the inoculation made no difference in the external appearance of the stalks. Of all the plants inoculated when a foot high, only those of strain A1237 showed black bundles. In the strains inoculated at the appearance of silks, the bundles of strains BR10 and L111 were

black a few inches away from the point of injury, but the stalks as a whole were normal in appearance. As shown in table 4, 410 platings were made in which there were 65 *Cephalosporium acremonium* cultures and 364 were free of fungi. Five and one-tenth per cent of the early inoculations and 34.5 per cent of the late inoculations were effective. It is interesting to note that in strain L111 26 out of 30 stalks were found to contain active cultures of the fungus, but the areas infected in the stalks were restricted to within 2 inches of the point of inoculation. The fungus did not grow beyond these areas, and very small areas within the stalk were damaged by the puncture and the action of the fungus.

TABLE 4.—Summary of data on inoculations made with a hypodermic needle and a spore suspension of *Cephalosporium acremonium* under field conditions at Madison, Wisconsin—season of 1930

Platings listed by strains	Inoculation method				
	Spore suspension July 12	Water check	Spore suspension August 1	Check	Totals
A1237					
<i>C. acremonium</i>	5	1	11	1	18
Free of fungi	93	20	32	148	303
BR10					
<i>C. acremonium</i>	—	—	21	—	21
Free of fungi	—	—	48	—	48
L111					
<i>C. acremonium</i>	—	—	26	—	26
Free of fungi	—	—	4	—	4

At Bloomington, Illinois, a large number of hybrid and inbred strains of corn were inoculated with a spore suspension of the fungus. This was done when the tassels began to show. Two plots were used. Treatments were alike, except that the checks in the first plot were not given the sterile distilled water injection. When mature, a large number of stalks were split open and examined (Table 5). In this series, including more than 5,000 plants, it was noted that *Diplodia zeae* attacked many stalks inoculated with a spore suspension of *Cephalosporium acremonium* and also those injected with sterile distilled water, entering through the wound made by the hypodermic needle. The stalks not attacked by *D. zeae* and that had been inoculated with *C. acremonium* showed no outward symptoms of disease at the end of the season. The drouth was severe and greatly reduced the yield of corn, but *C. acremonium* seemed to react on the plants in the same

way as it did at Madison, Wisconsin, where similar experimental plots were grown to maturity under normal soil-moisture conditions. No platings were made in this series, the experiment being designed to bring out the external symptoms, which have been described by other workers as being due to an inoculation with the fungus. No external symptoms of disease appeared and thus the experiment checked with other inoculation experiments in which the organism was recovered.

TABLE 5.—Showing the influence of inoculation with a spore suspension of *Cephalosporium acremonium* into corn plants grown at Bloomington, Illinois—season of 1930

Data on inoculation series and check series	Number of stalks examined		
	Plot No. 279	Plot No. 283	
	Inoculation	Inoculation	Control
Inoculated with <i>Cephalosporium acremonium</i>	1776	1785	—
Injected with sterile distilled water for control purposes	—	—	1774
Examined for black bundles by splitting open the stalks	712	897	839
Bundles blackened by <i>C. acremonium</i> inoculation only	351	277	—
Bundles blackened by an invasion of <i>Diplodia zeae</i> through wounds made by hypodermic needle	314	376	427
Bundles blackened as a result of the response of a number of strains to severe drouth conditions ¹	47	226	170
Bundles white, apparently normal, and not damaged by the sterile water injection	—	—	242
Average distance bundles were blackened due only to an inoculation of <i>C. acremonium</i> spores	7.8 in.	7.7 in.	—

¹ Under normal environmental conditions in which there is no soil-moisture deficiency, most of the strains recorded in this group do not have black bundles.

DISCUSSION

In this study it has been shown that the black-bundle condition of corn is found consistently in some inbred strains, occasionally in some, and is totally lacking in others. Strain A1237 is consistent in having some discolored bundles in every stalk. Strain L111 is consistently free of any discoloration of the bundles. Some inbred strains of corn have darkened bundles when grown under adverse environmental conditions, while, under normal growing conditions, no black bundles occur.

Since Reddy and Holbert (7) named the fungus *Cephalosporium acremonium* as the causal fungus of the black-bundle disease, a study was made of the occurrence of the fungus in the black bundles found in strain A1237 and in the occasional stalks of other strains showing this condition.

The results show conclusively that, in those inbred strains of corn studied, the presence of the fungus in the bundles is not a factor in bringing about the black-bundle condition, only 4 per cent of the bundles being infested with the fungus.

A histological study of black bundles showed that the cells, particularly those of the protoxylem and phloem, were filled with a dark, amorphous material, ranging in color from a light brown to black. This material was studied microchemically in both fixed and fresh sections. While the results are inconclusive, enough was learned of the nature of the substance to demonstrate that it contains, in part, pentoses.

The gum-like substance in the bundles was evidently either an environmental response of the plant or due to a hereditary factor in strains such as A1237, where it occurs in every stalk. An effort was made to produce the black-bundle condition in strains which were normally free of it. It was demonstrated that darkened bundles could be produced either by drouth conditions or an unbalanced nutrient condition in inbred strains, where they do not normally occur. Later, this was again demonstrated under field conditions by the inbred strain BR10. It is normally free of vascular discoloration, but showed many black bundles in each stalk when grown under the drouth conditions of the year 1930.

Since the fungus *Cephalosporium acremonium* was occasionally found in black bundles, several attempts were made to inoculate corn plants with the fungus. Seedlings were grown in infested soil. The roots of the primary root system were injured, and ten days later the fungus had penetrated several centimeters up the vascular bundles of these roots. However, at maturity only 10 per cent of the plants thus invaded were infested with the fungus in the vascular bundles of the stalks. Thus it is demonstrated that plants invaded by the fungus in the seedling roots may continue growth to maturity without the fungus penetrating to other portions of the stalk.

When spores of the fungus were hypodermically injected into the vascular bundles of various strains of inbred corn and the plants brought to maturity, no differences could be noted in any strain except A1237, which has black bundles consistently. In strain A1237, wounding by the hypodermic needle caused stunting, both in plants injected with sterile water and in plants inoculated with a spore suspension of the fungus. The fungus, in all strains except A1237, remained alive in the wounded bundles until the stalks had matured, but had progressed only a very short distance from the point of inoculation.

When cultured on artificial media, the fungus grew most vigorously on medium containing xylan isolated from corn cobs. In the vascular bundles of corn stalks free of the brown gum-like substance, the fungus made no ap-

preciable growth. In bundles where the gum was present the fungus grew vigorously, if able to penetrate through wounds to the gum-filled bundles. The results of the study suggest that *Cephalosporium acremonium* is not a vigorous pathogenic fungus, but rather is it a saprophyte that occasionally is able to penetrate through wounds to vascular bundles already partially filled with a gum-like substance offering food material to the fungus.

SUMMARY

Previous workers have described the black-bundle disease of corn as being caused by invasion of the vascular bundles by the fungus *Cephalosporium acremonium*. Abnormal external growth features were ascribed to hereditary factors, improper nutrition, and the invasion of the fungus *C. acremonium*.

In this study the blackening of the vascular bundles in certain inbred strains was found to be due to a deposit of gum-like material in the cells and the vessels of the bundle. In a small percentage of cases the fungus was present and found to be closely associated with the gum. Preliminary micro-chemical analysis of the gum indicates the presence of small amounts of pentoses, which serve as food for the fungus.

The deposit of gum in the vascular bundles was found associated with hereditary characters in one inbred strain of corn, as well as being influenced by environmental conditions. Black bundles were produced under controlled environmental conditions by limiting the water supply of plants of one strain of corn and by supplying an unbalanced nutrient solution to plants of another strain. Under field conditions, black bundles were observed to occur in some strains during the severe drouth of 1930. In another strain, soils deficient in phosphorus were found to cause black bundles.

Inoculation experiments with corn seedlings of the several inbred strains under observation did not result in infection. Planting infected seed did not result in the establishment of the fungus in the growing plant. Older plants, nearing maturity, from strains of corn having vascular bundles filled with a gum-like substance were found to be invaded by the fungus. Strains of corn having bundles free of the gum-like substances were not invaded by the fungus. Infection of those strains from which the fungus was cultured was found to have taken place after the corn stalks had tasseled out and following severe root injury. In none of the experiments made was the fungus found to be an active pathogen invading normal vascular bundles through uninjured root systems.

DEPARTMENT OF PLANT PATHOLOGY,
CALIFORNIA STATE DEPARTMENT OF AGRICULTURE,
SACRAMENTO, CALIFORNIA.

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LEAF BLIGHT OF CHINA ASTER CAUSED BY *RHIZOCTONIA SOLANI*¹

ARNOLD J. ULLSTRUP

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INTRODUCTION

During the autumn of 1935 a considerable number of China aster plants (*Callistephus chinensis* Nees) growing under greenhouse conditions at Princeton, New Jersey, exhibited a severe blighting of the leaves. A fungus identified as *Rhizoctonia solani* Kühn was isolated from the diseased leaf tissue, and its pathogenicity was established by pure-culture inoculation.

While it is well known that *Rhizoctonia solani* has a wide host range, and that plants may be attacked at different stages of development (8), a study of the literature indicates no report of the fungus as causing a leaf blight of China aster. Duggar and Stewart (2) have reported *R. solani* responsible for stem lesions on mature China aster plants, and Peltier (8) has shown the fungus to be capable of causing damping off of the seedlings of this host. Direct attack of leaves by *R. solani* has been pointed out, by both Duggar (1) and Matsumoto (6), to be infrequent. Examples of leaf diseases caused by this fungus have been described by Stone and Smith (10) for lettuce, and by Wellman (11) for cabbage.

Whether or not this isolate of *Rhizoctonia solani* was peculiar in its ability to attack the leaves of China aster was not known. A study was, therefore, undertaken to compare this isolate with others of the same species, but collected from different hosts. The present paper deals with the results of these comparative studies.

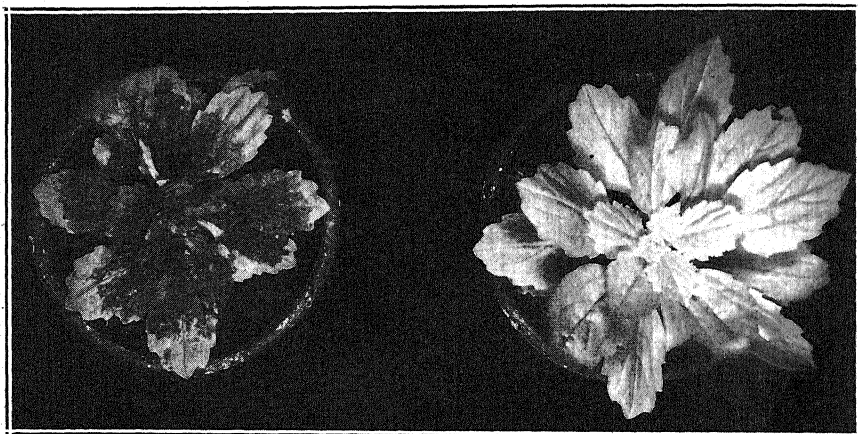
THE DISEASE

The disease was first noticed in the early autumn of 1935 on 2- to 4-month-old plants during and shortly following an extended period of warm, cloudy weather. As the season advanced and the temperature lowered, a decrease in incidence of blighting became evident.

Symptoms of the disease first appeared on the lowermost leaves, which had opportunity of coming in contact with the soil. Affected tissues were characterized by a clearly defined, water-soaked area that progressed rapidly over the leaf. Within a short time the infected area collapsed and became

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flaccid, turning from a deep green to a dead brown. More or less concentric zones of alternating light and dark brown were often observed spreading from an infection center. Following collapse of the leaf blade, the infection spread down the petiole to the stem, where other leaves became involved. Severely blighted leaves often showed scattered strands of coarse mycelium traversing their surfaces. Under favorable conditions for development of the disease, plants frequently were killed within a period of a few days (Fig. 1).



Photographed by J. A. Carlile.

FIG. 1. The China aster plant on the left shows severe infection by *Rhizoctonia solani*. The plant on the right is healthy.

EXPERIMENTAL

In order to determine whether the isolate obtained from diseased China asters was similar to or markedly different from isolates of *Rhizoctonia solani* from other hosts, a number of comparative experiments were undertaken. The experiments consisted of studies on growth rates at different temperatures, growth and cultural behavior on various agar media, cross inoculations, and modes of penetration. An outline of the history of the 5 isolates of *R. solani* employed is given in table 1.

Temperature Relationships. Each isolate was grown in triplicate on plates of potato-dextrose agar at each of 7 different temperatures. Mycelium used in seeding the plates was cut in equal amounts from the marginal growth of 4-day-old colonies grown on the same medium at the respective temperatures. In the case of those isolates that failed to grow at the extreme temperatures, mycelium used to seed the plates was taken from colonies grown at the next closest temperature. Daily measurements were taken of the diameters of the colonies for 3 succeeding days after placing the cul-

TABLE 1.—*History of isolates of Rhizoctonia solani used in the experiments*

Isolate	Host from which isolated	Geographical origin of isolate	Date of isolation
A-1	China aster	Princeton, N. J.	1935
P-1	Potato (Sclerotium)	"	"
SB-1 ^a	Sugar beet	Minnesota	1934
SB-2	"	"	1930
SB-3	"	Michigan	"

^a The strains isolated from sugar beets were furnished to the writer through courtesy of Dr. E. L. LeClerc.

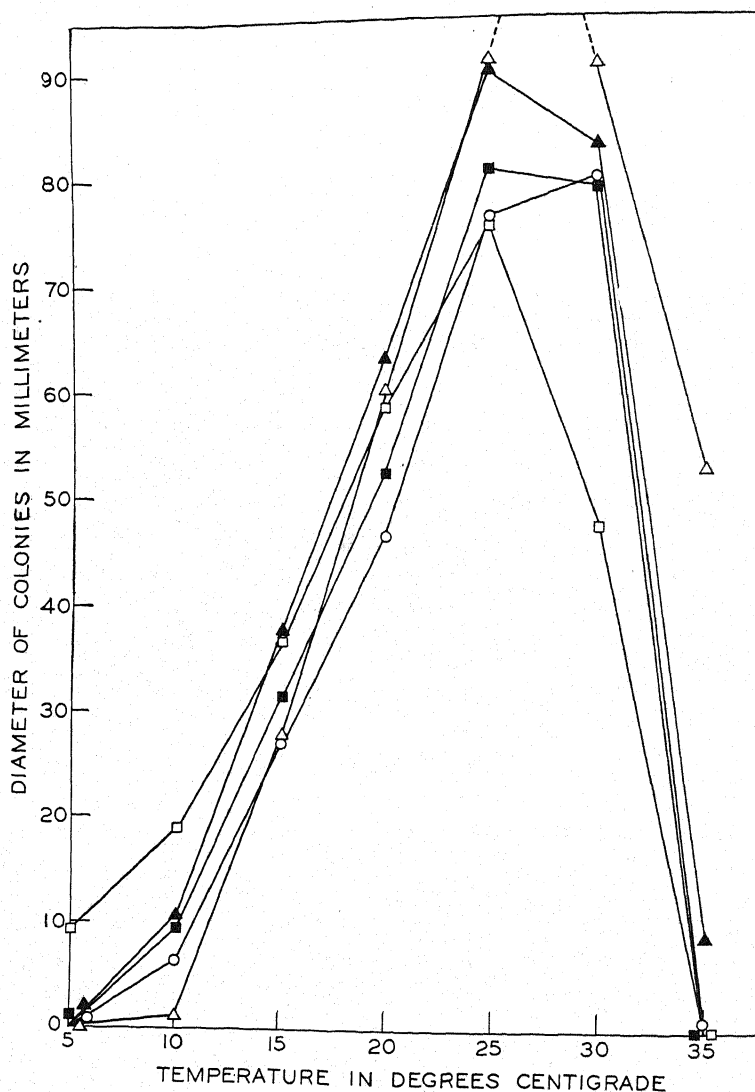
tures at the various temperatures. Growth curves for the isolates at the end of the 3rd day at the different temperatures are shown in figure 2, and are based on the average of 3 replications of the experiment.

Isolate A-1 showed no growth at 5° C., very little at 10° C., and an optimum growth temperature between 25° and 30° C. The outstanding feature is the amount of growth produced by this isolate at 35° C. Isolate SB-3, although making much better growth at the lower temperatures than A-1, was the only other culture to show appreciable growth at 35° C. Isolates SB-1 and SB-2 showed a close similarity in temperature relationship, and were intermediate in this respect between P-1 at the low temperatures, and A-1 at the higher temperatures. The culture P-1 grew better at comparatively low temperatures and showed a pronounced reduction in colony diameter above 25°.

Growth on Different Agar Media. In this experiment the isolates were grown in triplicate at room temperature (22°–24° C.) on the following agar media: malt, prune, cornmeal, lima-bean, potato-dextrose, oatmeal, Richard's, and Coon's.² Mycelium used to seed the plates was cut in equal amounts from the marginal growth of 4-day-old colonies growing on each of the media. The diameters of the colonies were measured at 24-hour intervals for a period of 3 days.

The data in table 2 show Coon's agar to be the most favorable medium

² The malt, prune, cornmeal, and Lima-bean media were prepared "Difco" agars. Potato-dextrose agar was made as follows: 200 g. of peeled potatoes were steamed for ½ hour in 500 cc. distilled water. To the filtered potato juice 20 g. of dextrose were added, and 500 cc. of melted 4 per cent agar were then mixed with the potato juice to bring the final agar concentration to 2 per cent. The mixture was made up to volume (1 liter) and autoclaved 20 minutes at 20 lbs. pressure. The synthetic media were of the following composition: Richard's solution agar: sucrose, 50 g.; magnesium sulphate, 2.50 g.; monobasic potassium phosphate, 5.00 g.; potassium nitrate, 10 g.; agar, 20 g.; distilled water, 1000 cc. Coon's medium agar: sucrose, 7.20 g.; dextrose, 3.60 g.; magnesium sulphate, 1.23 g.; monobasic potassium phosphate, 2.72 g.; potassium nitrate, 2.02 g.; agar, 20 g.; distilled water, 1000 cc.



Photographed by J. A. Carlile.

FIG. 2. Graph of growth curves of 5 isolates of *Rhizoctonia solani* at 7 different temperatures at end of 3 days.

△ = Isolate A-1

□ = Isolate P-1

○ = Isolate SB-2

▲ = Isolate SB-3

■ = Isolate SB-1

for the growth of isolate SB-3. This medium also supported the best growth in the case of isolate A-1. While not expressed in the table, readings of the colony diameters of this isolate on Coon's agar on the 1st and 2nd days

were greater than on the other media. Oatmeal agar proved to be the most favorable medium for the growth of cultures SB-1, SB-2, and P-1. In this experiment, as well as that on temperature relations, a similarity in behavior was found between isolates A-1 and SB-3 and between the isolates SB-1 and SB-2.

TABLE 2.—*Growth in millimeters of the 5 isolates of Rhizoctonia solani on different agar media*

Isolate	Colony diameters in millimeters on 8 agar media at the end of 3 days							
	Malt	Prune	Corn-meal	Lima-bean	Potato dextrose	Oatmeal	Richard's	Coon's
A-1	85	57	78	90 + ^a	90 +	90 +	65	90 +
P-1	48	46	60	77	80	90 +	40	52
SB-1	44	20	25	63	58	90 +	29	38
SB-2	45	33	42	82	64	84	30	44
SB-3	63	72	79	90	82	81	80	90 +

^a The + sign indicates that the colonies had overgrown the entire agar surface.

Comparisons were made of the cultural behavior of the 5 isolates *Rhizoctonia solani* to determine possible similarities or differences between them. The following is a description of the cultural characters of each isolate grown for 7 days at 22°–24° C. on potato-dextrose agar:

- A-1. Sclerotia irregular in size and shape, sparse over the surface of the colony, but abundant about the edge and in contact with glass wall of the Petri plate. Aerial mycelium abundant, closely compressed to surface of agar, and showing radiating lines of mycelial growth. Color of colony: olive buff.
- P-1. Sclerotia irregular in size and shape, aggregated in clumps in center of colony but diminishing in abundance towards the edge. Aerial mycelium moderately abundant and woolly in appearance. Color of colony: olive brown, fading in intensity towards edge.
- SB-1. Sclerotia irregular in size and shape, evenly distributed over entire colony. Aerial mycelium abundant and woolly in appearance. Color of colony: sepia brown, of equal intensity over entire colony.
- SB-2. Sclerotia irregular in size and shape, evenly distributed over the surface of the colony. Aerial mycelium abundant and woolly in appearance. Color of colony: tawny olive.
- SB-3. Sclerotia very sparse and small. Aerial mycelium abundant, closely compressed to surface of agar and showing radiating lines of growth. Color of colony: pale olive buff.

In general, the isolates A-1 and SB-3 show a close similarity in cultural behavior, as do also isolates SB-1 and SB-2. Isolate P-1, although resembling SB-1 and SB-2 in many respects, differs markedly in the abundance and aggregation of sclerotia.

Cross inoculations. Young plants of China aster, sugar beet (*Beta vulgaris* L.) and potato (*Solanum tuberosum* L.) were inoculated with each of the 5 isolates by placing pieces of mycelium of equal size on the leaves and stem. Inoculum was held in place by covering with pieces of moist absorbent cotton. Controls consisted of plants to which only the moist absorbent cotton was applied. The plants were placed in a moist chamber for 48 hours, after which they were removed to a greenhouse bench. Two days later, observations were made on the pathogenicity of the isolates on the 3 hosts. A summary of the results is given in table 3.

TABLE 3.—*Relative pathogenicity of the 5 isolates of Rhizoctonia solani on 3 hosts*

Isolate	Relative degree of pathogenicity		
	Sugar beet	China aster	Potato
A-1	Very severe infection	Very severe infection	Moderate infection
P-1	No infection	No infection	No infection
SB-1	Moderate infection	Severe infection	Moderate infection
SB-2	“ “	“ “	“ “
SB-3	Severe infection	“ “	“ “

Each isolate was relatively uniform in its behavior on a given host. No infections occurred in the noninoculated controls. The aster isolate, A-1, was unquestionably the most pathogenic of all cultures employed. Sugar beet plants inoculated with this isolate were characterized by severe infection that spread from the point of inoculation and involved most of the aboveground parts. The same general condition held for China aster plants inoculated with this isolate. Potato plants, while showing distinct symptoms, proved to be much more resistant to all isolates than did the other 2 hosts.

The potato isolate, P-1, was found to be nonpathogenic under the conditions of the experiment.

Of the cultures SB-1, SB-2, and SB-3, the latter was slightly more pathogenic on sugar beets and China asters than the other 2.

Penetration Studies. During the course of the investigation, the question arose as to whether or not the nonpathogenic isolate, P-1, penetrated the tissue of the inoculated plant. It was desired also to determine the modes of penetration of the other isolates.

The plants used in these studies were China aster, sugar beet, and begonia (*Begonia semperflorens* Link and Otto). Since the stomata of this species

of begonia are found only on the lower surface of the leaves, it afforded a means of studying penetration both in the absence and in the presence of these natural openings.

The leaves were inoculated on both surfaces and the plants placed in a moist chamber. In addition, leaves that had been wounded with a series of pin punctures also were inoculated. At 24-hour intervals, leaves, each of which was inoculated with a single isolate, were removed and pieces of tissue cut out from around the place of inoculation. The pieces of tissue were placed in a solution of equal parts of absolute alcohol and glacial acetic acid to remove the chlorophyll, then cleared in lactophenol, and finally stained in lactophenol to which acid fuchsin had been added.

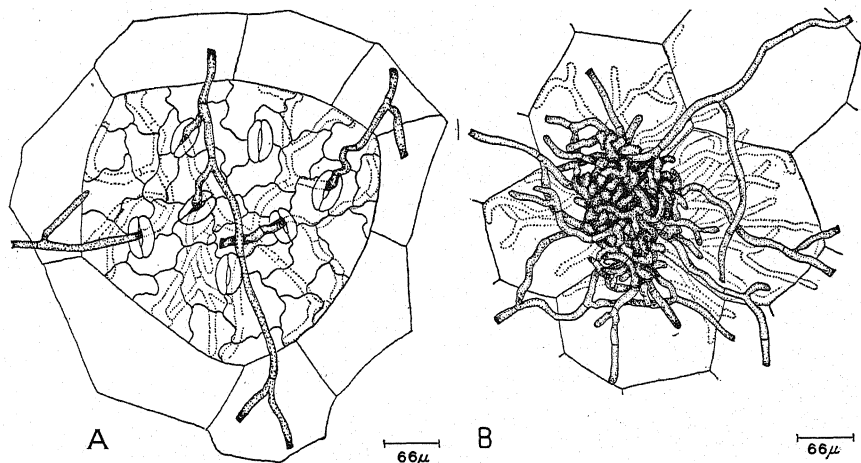


FIG. 3. A, Hyphae penetrating through stomata on lower surface of Begonia leaf. This was found to be the characteristic mode of penetration of cultures SB-1 and SB-2. B, "Infection cushion" on upper surface of Begonia leaf, by means of which hyphae of cultures A-1 and SB-3 are able to penetrate cuticularized epidermal cells.

The material fixed 24 hours after inoculation showed no evidence of penetration by any of the fungus cultures. After 48 hours, infection was macroscopically evident on leaves inoculated with isolates A-1 and SB-3. Microscopic observation showed the mycelium traversing the surface of the leaves and small stromatic areas of knotted fungus strands distributed among the hyphae (Fig. 3, B). The stromatic areas or "infection cushions" have been described already by Duggar (1) as playing an important rôle in effecting penetration.

Initial penetration by the isolates A-1 and SB-3 takes place immediately beneath infection cushions and seems to be a mechanical process. The infection cushions appear to act as a fulcrum against which a force is exerted that is sufficient to allow hyphae to penetrate uninjured, cuticularized epi-

dermal cells. Infection cushions were formed on the lower as well as the upper surface of all inoculated leaves, regardless of the location of stomata.

The exact means by which the infection cushions are held on the surface of the host, to permit a force to act on them without changing their position, was not determined. No cementing material was ever observed around the cushions. Furthermore, the cushions could easily be lifted from the leaves by means of a fine glass needle, suggesting that their weight may be sufficiently great to balance the pressure exerted by the penetrating hyphae. Swelling of the ends of the hyphae in contact with the cell walls, and bulging of the latter in the direction of the force exerted, suggest that the progress of the fungus within the host tissue is largely a mechanical process.

The inoculated plants were held in a moist chamber for several days and development of infections followed. Wounded and nonwounded leaves of plants inoculated with isolates A-1 and SB-3 were heavily infected at the end of 5 days. Nonwounded *Begonia* leaves, inoculated on their upper surfaces with isolates SB-1 and SB-2, showed no infection at the end of 7 days. Wounded leaves of all plants, nonwounded leaves of sugar beet and China aster, and nonwounded leaves of *Begonia* inoculated on their lower surfaces with SB-1 and SB-2 showed marked symptoms of infection within 5 days.

Leaves inoculated with isolates SB-1 and SB-2 showed evidence of penetration and infection after 48 hours. Penetration occurred, however, only through the stomata (Fig. 3, A). In no instance was penetration found to take place through the uninjured cuticle. Infection was established by all isolates, except P-1, in leaves wounded by pin punctures. The hyphae of P-1 were observed growing through and about cells killed by the punctures, but subsequent penetration of living cells was never observed.

DISCUSSION

The formation of infection cushions and their rôle in effecting direct penetration appear to be only partially responsible for the high degree of pathogenicity shown by isolates A-1 and SB-3. That the ability of a given culture to penetrate directly does not seem to be the sole criterion for determining pathogenicity is suggested by the fact that certain cultures, namely, SB-1 and SB-2, do not form these infection cushions, but gain entrance into the living tissue only through stomata. Furthermore, the virulence of a culture appears to depend on more than simple penetration, since in the case of isolate P-1, where hyphae entered the host through wounds, subsequent penetration and parasitism of living cells did not take place. It is probable that in nature the most highly pathogenic cultures of *Rhizoctonia solani* penetrate directly and are not dependent on wounds or stomata as avenues of entrance into their hosts.

In addition to their very marked pathogenicity and mode of host penetration, the cultures A-1 and SB-3 could be differentiated from cultures P-1, SB-1, and SB-2 on the basis of cultural characters, growth on agar media, and temperature relationships. Cultures A-1 and SB-3 could be distinguished from each other primarily on the basis of their growth rates at 35° C.

Although isolates of *Rhizoctonia solani*, such as A-1, may have very well defined characteristics that serve to distinguish them one from another at a given time, the continued stability of such characters seems somewhat doubtful. From time to time during the course of the work, all isolates gave rise to one or more sectorial variants differing in cultural behavior from the original parent culture. The variants, however, were not studied in any detail, and whenever such sectors arose the original culture was segregated and maintained separately. The fact that many different types of cultures of *R. solani* are encountered on original isolation, suggests that variants may arise as frequently under natural conditions as in artificial culture.

The presence of definite strains within the species *Rhizoctonia solani* has been recognized by several investigators, namely, Matsumoto (6), Edson and Shapovalov (3), Rosenbaum and Shapovalov (9), Gratz (4), and LeClerc (5). These strains have been distinguished on the basis of pathogenicity, and on their physiological and cultural characters while being maintained on artificial media.

Peltier (8), and Monteith and Dahl (7), on the other hand, have pointed out that the fungus is highly variable and specialization towards particular hosts is not sufficiently distinct to warrant a definite classification of strains.

In any fungus species like *Rhizoctonia solani*, in which the mycelium is multinucleate and uninucleate spore forms are not produced in artificial culture, the possibility of the existence of a heterocaryotic thallus cannot be overlooked. Where heterocaryosis exists in the original isolates, attempts to delimit them into highly specialized strains of stable characters would appear to present many difficulties.

SUMMARY

A leaf blight of China aster caused by *Rhizoctonia solani* is reported and symptoms of the disease described.

The culture of the fungus, A-1, isolated from diseased aster leaves, has been shown to differ in its physiology, cultural characters, pathogenicity, and mode of host penetration from certain other cultures (SB-1, SB-2, and P-1) of the same species. Similarity in certain characters was shown to exist between the culture A-1 and the culture SB-3 isolated from sugar beets.

No marked host specialization was found to exist among any of the isolates tested.

The cultures A-1 and SB-3 produced infection cushions on the surface of the host which function in direct penetration of the cuticle. Cultures SB-1 and SB-2 were observed to penetrate only through stomata without the intervention of infection cushions. The culture P-1 did not penetrate the living tissue of any of the plants inoculated, even though the latter had been wounded and the hyphae were in contact with the exposed noncuticularized tissue.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY

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ANALYSIS OF TYPICAL PLANT DISEASES FROM THE QUARANTINE STANDPOINT¹

W. A. McCUBBIN

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In estimating the worth of plant quarantines as a means of excluding injurious plant diseases in a permanent way, the logical procedure would be to get together a comprehensive body of information on the foreign parasites against which quarantine action would be directed; then, by studying these in the light of the quarantine measures available for protection, it should be possible to arrive at some general evaluation of the worth of a plant quarantine system for exclusion purposes.

Unfortunately, such an orderly method of approach is seriously hampered at the outset by lack of requisite information on the foreign parasites directly concerned. In many cases the casual agent is new, or has been only slightly studied; references in literature are scanty at times; language difficulties interfere with complete understanding; and the record is too often incomplete from the plant quarantine standpoint. It is a not uncommon experience to encounter a descriptive article that may be highly satisfactory to the mycologist but gives no hint of the very points necessary in appraising the disease from the quarantine viewpoint.

Because of these and other difficulties incident to a direct approach to the problem, an indirect method has been adopted. An attempt has been made to subject to analysis a selected list of 200 more or less typical plant diseases, so as to bring out the relations of their life habits and hosts to accepted quarantine procedure. If we may then assume that these diseases represent in a general way the perplexing field of plant diseases with which quarantine measures would have to deal, the conclusions that may be drawn from them as to the value of quarantines for exclusion purposes ought to hold fairly well in regard to the whole field of plant diseases.

Recognizing that in this indirect method of approach the value of the conclusions depends wholly on the faithfulness with which the substitute list represents the whole field of plant diseases, considerable care has been taken with the examples chosen. In addition to giving consideration only to diseases of outstanding character, an effort has been made to so select the examples that they may be typical, not only of the general types of disease commonly met, such as rots, wilts, rusts, leaf spots, etc., but also so that the list would include a considerable diversity of parasitic species and a varied

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range of hosts. Although most of the hosts because of this method of selection are plants of some economic importance, the economic status of the host has been entirely disregarded in the analysis and the appropriate quarantine action has been derived in each case solely from the biological relationships of host and parasite.

For the sake of simplicity exclusion is here thought of ideally in connection with some sufficient natural barrier, such as a sea, desert, or mountain range, across which materials must be transported by human agency to effect distribution of the disease. The question of exclusion from an adjacent portion of the same land area is not specifically dealt with, though, to the extent that this more difficult and complicated problem involves the transport of disease-carrying materials, the conclusions will be generally applicable.

In this analysis there had to be faced the possibility of the transport of spores or other living stages of parasites on all sorts of commodities other than the hosts themselves and articles directly connected with these hosts. It is quite possible, for example, that spores of *Graphium ulmi* might be carried on such things as azalea plants, or corn smut spores on rye straw packing. But, short of stopping all international interchange of goods, there appears to be no way of avoiding this risk; it simply must be accepted. Consequently, there has been adopted a conservative attitude that disregards incidental spore transportation unless the host material under consideration has had special or definite opportunity to become a carrier. From this viewpoint potato seeds are regarded as free from spores of *Corticium*; but bean seeds, very likely to become contaminated by spores of *Cercospora cruenta* in threshing operations, are treated as carriers of that fungus.

In this study four types of quarantine action are recognized—*embargo*, *detention*, *disinfection*, and *inspection*. The addition of unrestricted entry gives us five methods of disposal for imported plant materials. *Embargo*, as the term is universally understood, involves an absolute prohibition on the entry of specific materials. *Detention* is here considered to mean a delay in release during which period the plant materials are held under conditions precluding the escape of parasites, and under careful observation, until freedom from the parasite is assured, or the organism has been eliminated, or until the development of infection compels destruction. Detention must be interpreted as including all holding conditions appropriate for the particular disease concerned, and might range from daily scrutiny in a closely constructed greenhouse to mere field culture under proper observation. It would likewise include the sorting, inspection, and disinfection which would naturally be associated with this means of obtaining disease-free plant products. *Disinfection* also is used in a broad sense to include the considerable number of treatments regarded as effective in freeing various plant materials from their living parasites. Inspection methods and safe holding pro-

visions are of course natural elements in such procedure. *Inspection* is here used in its commonly accepted sense, with the meaning that the plant materials will be examined carefully by a competent official, and if objectionable disease conditions be found, appropriate action will be taken, such as refusal of entry, requirement of treatment, or destruction of the material. Inspection throughout this analysis is considered as a distinct quarantine function only when it alone is depended on as a means of protection; when used as an adjunct to detention or disinfection it is regarded merely as a part of that procedure.

It may be observed that certification of products in the country of origin has not been accorded a sixth place among the quarantine methods at our disposal. This feature was not omitted out of any disrespect for a highly important element in the world's protective arrangements. But, where complete exclusion of a disease is aimed at, could certification, as customarily practiced, be depended on as a sole and only means of protection? Is there anywhere a system of certification so accurate and infallible that it could be trusted to the utmost in the matter of protecting another country's vital interests? No doubt in a few special cases such confidence would be justified, but over the general field of diseases we are forced to conclude that certification, however valuable it may be otherwise, can be considered only as a helpful auxiliary to exclusion measures, or as a partial substitute for them.

The accompanying tabular statement presents separately the indicated quarantine action for seeds, propagating materials other than seeds, and commercial products. It is believed that this division of plant imports enables us to grasp the quarantine relationships with a closer approach to actuality than would any other method. Seeds as a group exhibit characteristic differences from plants, bulbs, or roots, in their concentrated volume, a considerable natural freedom from infection, the usually dormant condition of organisms present, and inability to withstand treatment. Commercial products, on the other hand, develop at times special problems as compared with seeds and other plant propagating materials, because of such factors as their large volume, wide distribution, the presence of active stages of parasites, susceptibility to treatment injury, and the undesirability of using poisonous fungicides on various food products. The results of the analysis seem to substantiate the correctness of this classification. One could perhaps reasonably expect still more fruitful results if these categories were subdivided further, but that refinement is beyond the scope of the present simple analysis.

Turning attention to the tabulated results, it is seen that the 200 diseases fall into 16 categories representing as many types of disease. By this segregation on a roughly symptomatic rather than systematic basis, it was

hoped to uncover any possible differences among these disease types. Any marked differences would have considerable practical significance in actual quarantine procedure. Within each disease type were assembled as varied a series of causal agents as possible.

Glancing now at the summarized totals it is apparent that for 199 seeds (including fern spores) embargo is scarcely needed (0 cases); detention would be required in but few cases (8); disinfection seems to be highly important (116); inspection has but little value (1); and unrestricted entry could be permitted in an encouragingly large proportion (74).

In materials for propagation other than seeds, which occur in 132 of the 200 examples, the picture is not so favorable. It is true that in only 12 cases embargo would seem to be necessary; but 86 would require detention, and in only 29 the more feasible disinfection procedure could be relied on. For a disappointingly small number, inspection (4), and unrestricted entry (2), are seen to be satisfactory.

Commercial products present a most discouraging feature in the large proportion of cases where embargo is indicated if exclusion is sought. One hundred ninety-four of the examples involve products that might be of this type and it is rather disturbing to note that embargo is indicated as a necessary measure in 144 of these instances. Of these 144, 20 cases involve cut flowers, 20 concern straw, important from the quarantine point of view on account of its universal use as packing, and 9 deal with hay. Eighty-three however relate to grain, fruits, vegetables, and similar products entering into commerce in often large quantities. In 10 cases the product affected may be classed as of minor importance.

Even if we disregard cut flowers and the 10 items of minor importance, there still remain 112 cases out of 194 where an exclusion program would have to consider embargo; and it is probable that this proportion would be further increased by the necessity of resorting to embargo in a good many cases where disinfection is indicated as a correct enough biological procedure, but where its use would be accompanied by troublesome difficulties. In this connection one might mention the disinfection of table potatoes to eliminate *Rhizoctonia*, or of wheat to destroy the internal stage of bunt, *Tilletia tritici*. The outlook for disinfection for these products is, likewise, not very promising, since there are only 31 instances where it could be used effectively. Inspection makes a slightly better showing than in the other two categories, but the mere 12 cases where inspection alone is deemed effective give this function a very unimportant rank. In 56 cases at least some article of commercial type could be allowed unrestricted entry with safety.

As far as these 200 diseases can be considered representative, their analysis indicates that in any serious effort to exclude plant diseases three outstanding quarantine functions must be called into frequent use. In the case of

seeds disinfection would be of preeminent value; detention would be highly important for other propagating materials; and for commercial products embargo would have most frequent use. Disinfection seems to have a rather limited application in the case of propagating materials other than seeds and for commercial products, while detention methods appear to be of quite secondary importance for seeds. The very low percentage of cases in all three categories where inspection alone can be relied on for disease-exclusion purposes suggests that this phase of quarantine activity should not be given undue importance in a system of quarantine aimed at exclusion of diseases.

If we attempt to translate the results of this analysis into terms of quarantine policy, the most disconcerting aspect seems to be the large scale on which embargo of commercial products would have to be considered as the price of adequate disease exclusion. It is true that the actual extent of embargo might be less than the recorded percentage of cases would indicate, since the embargo in many of the cases cited is but a partial one. On the other hand, it is almost certain that an administration would in some cases be driven by necessity to resort to embargo where a less drastic procedure is biologically correct, but, for one reason or another, can not be made effective. We must take into consideration also the very important fact that many diseases involve a much wider host range than the single one that, for the sake of simplicity, has here been associated with the parasite. Less than 60 of the 200 diseases listed are restricted to one host or to a few closely related plants. The remaining 140 have such numerous hosts that any attempt to use embargo freely and consistently throughout this group must promptly and profoundly reduce imports. In over 40 cases the number of hosts is so great that embargo for these is almost out of the question.

It would appear, therefore, that the chief limiting factor in setting up an ideal plant-protection system will be found in connection with commercial products rather than in seeds and other propagating materials. We are sometimes inclined to stress the seriousness of the risk in propagating materials in the matter of disease introduction, and in one sense this attitude is correct. For reasons obvious to every plant pathologist these materials are extremely dangerous, at least potentially. But this analysis seems to indicate that, in a broad way, methods are available that can be employed effectively to provide for safe entry of a very generous proportion of these necessary means of propagation. An efficient protective system, liberal enough for a country's healthy horticultural development, and well within the limits of public tolerance, is seemingly quite attainable for seeds and propagating stock, even if the ideal cannot always be reached. But in connection with commercial products this hopeful outlook is lacking. Any system of protection aiming at a complete program of disease exclusion based on correct biological principles would be compelled at the outset to reduce imports of

commercial plant products to a decidedly embarrassing if not dangerous degree, and probably much beyond the point of popular tolerance.

In this predicament a compromise system would appear to be inevitable. The ideal itself is beyond the reach of every country with considerable foreign interests. How close to perfection can the compromise be established? We may suppose that the standard will vary for different situations. It is quite understandable that a disease-exclusion policy that might serve for an essentially industrial nation importing large quantities of needed plant products would be considered quite inadequate for a country devoted primarily to agriculture, or that exclusion measures judged to be adequate for a one-crop nation would fall far short of the protection it would be profitable to establish in a land with many highly diversified crops.

In the average case it is probable that the compromise would settle down into a policy involving at the least an earnest attempt to exclude specific diseases likely to be destructive to crops of national importance, and in addition, an endeavor to keep out as many other diseases as possible by exclusion measures that may be effected without undue disturbance to the national economy.

SUMMARY

An analysis of 200 typical plant diseases from the quarantine standpoint indicates the quarantine action (embargo, detention, disinfection, inspection, and unrestricted entry), which would be necessary for seeds, propagating materials other than seeds, and commercial products, of the chief host, if exclusion of these diseases is aimed at. Summarized results indicate that embargo is scarcely needed for seeds, would have a minor use for other propagating materials, but would be necessary on a large scale for commercial products. Comparatively few seeds would require detention, but for other propagating materials detention would be freely used. Disinfection, the important procedure for seeds, is only moderately useful for other propagating materials and for commercial products. Inspection of itself has in all three categories a very low rank for exclusion purposes. Many seeds, but very little other propagating material, are safe for unrestricted entry; a rather limited number of commercial products are safe for such entry. The limiting factor in an ideal exclusion system appears to lie in the field of commercial products rather than in propagating materials; for the latter effective quarantine protection is practically attainable, but the need for excessive embargo in the case of commercial products tends to restrict quarantine action to a compromise system far short of perfection.

TABLE 1.—(Continued)

No.	Host	Causal organism	Seeds				Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection
VIRUS TROUBLES—(Continued)																
186	Peach	(Phony Peach)					x		x							x
187	Peach	(Yellows)					x		x							x
188	Potato	(Leaf Roll)					x		x							x
189	Raspberry	(Leaf Curl)					x		x							x
190	Sugar cane	(Mosaic)					x		x							x
191	Tobacco	(Mosaic)					x		x							x
192	Tomato	(Streak)					x		x							x
193	Wheat	(Rosette)					x		x							x
DISEASES DUE TO NEMATODES																
194	Beet	Heterodera schachtii					x		x							
195	Chrysanthemum	Aphelenchoides ritzema-bosi					x		x							
196	Clematis	Heterodera marioni					x		x							
197	Ferns	Aphelenchoides olesistius					x		x							
198	Narcissus	Anguillulina dipsaci					x		x							
199	Strawberry	Aphelenchoides fragariae					x		x							
200	Wheat	Tylenchus tritici					x		x							

INTERSPECIFIC TRANSFER OF A GENE GOVERNING TYPE OF RESPONSE TO TOBACCO-MOSAIC INFECTION¹

FRANCIS O. HOLMES

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The hybrid produced by crossing *Nicotiana rustica* L. and *N. paniculata* L. was first studied in 1761 by Kölreuter (7), who showed that repeated pollination of *N. rustica-paniculata* hybrids with pollen from *N. paniculata* resulted in a succession of generations of increasing fertility from the almost completely self-sterile F_1 to a fully self-fertile *N. paniculata*-like form. Kölreuter thus demonstrated that it was possible to obtain what appeared to be the species *N. paniculata* from *N. rustica* by repeated use of pollen alone. Other genetic studies of this hybrid, culminating in a series of papers emphasizing its cytological aspects (2, 8, 9, 10), have appeared since that time. Recently it was shown that specific genes control certain disease responses in the genera *Capsicum* and *Nicotiana* (4). A dominant gene *L* (virus-localizing) was found to confer immunity from systemic attack of tobacco-mosaic virus by localizing this virus in necrotic primary lesions in the pepper, *Capsicum frutescens* L. Within this species the gene was transferred from the varieties Tabasco and *minimum* to large-fruited strains by means of intervarietal crosses. Somewhat similar dominant genes were found in certain species of *Nicotiana*. One of these, in *N. rustica*, was studied in the first generation of the hybrid with *N. paniculata* (4, pp. 996-997). The hybrid *N. paniculata* \times *N. rustica* showed a necrotic type of response to infection with tobacco-mosaic virus, resembling in this respect the pollen parent, *N. rustica*. In young plants, infection was followed by systemic necrosis; in older plants, virus was localized in necrotic primary lesions. The hybrid showed no signs of the mottling, with attendant formation of enations, characteristic of the seed parent, *N. paniculata* (5).

This paper is concerned with a succession of backcrosses of the hybrid *Nicotiana paniculata* \times *N. rustica* var. *jamaicensis*. The production of this series involved repeated use of *N. paniculata* pollen, as well as retention only of plants that responded to infection with tobacco-mosaic virus by production of necrotic lesions. Subsequent selfing of plants that were heterozygous for the gene determining the necrotic type of response, and that resembled the species *N. paniculata* in appearance, gave rise to homozygous necrotic-type plants.

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EXPERIMENTS

From F_1 plants of constitution *Nicotiana paniculata* \times *rustica* a series of 5 generations was produced by application of *N. paniculata* pollen. Plants in the last 3 of these generations proved fertile to their own pollen, and were selfed. The following observations were made on ratios of necrotic-type to mottling-type individuals.

In the first of the backcross generations, 119 plants were grown and inoculated with tobacco-mosaic virus. Although these plants displayed great variability of leaf type and habit, only 2 kinds of response to infection were manifested. These were production of necrotic lesions, as in the F_1 hybrid, and mottling, as in *Nicotiana paniculata*. The ratio of necrotic-type to mottling-type plants was 60:59. In this backcross generation, therefore, a 1:1 ratio was obtained. Several of the necrotic-type plants were then pollinated with pollen from the mottling-type species, *N. paniculata*. In the second backcross generation, seed for which was obtained with difficulty from 2 plants, not a 1:1 but an approximate 2:1 ratio between necrotic and mottling types appeared in each of 3 sets. These 2:1 ratios were unexpected, and no entirely satisfactory explanation can be given for them with the evidence available at present. The practice of applying pollen of

TABLE 1.—*Ratios of necrotic-type to mottling-type plants in F_1 and 5 backcross generations of Nicotiana paniculata-rustica hybrids. All backcross generations were obtained by pollinating necrotic-type plants of a preceding generation with pollen from N. paniculata*

Generation	Necrotic-type plants	Mottling-type plants	Totals	Observed ratios
$F_1 = N. paniculata \times rustica$...	32	0	32:0	1:0
1st backcross	60	59	60:59	1.01:0.99
2nd backcross	37	18	128:68	1.96:1.04
“ “	65	34		
“ “	26	16		
3rd backcross	100	95	204:196	1.02:0.98
“ “	50	54		
“ “	54	47		
4th backcross	57	52	188:178	1.03:0.97
“ “	62	56		
“ “	69	70		
5th backcross	26	38	163:158	1.02:0.99
“ “	29	33		
“ “	35	32		
“ “	34	29		
“ “	39	26		

N. paniculata to emasculated flowers of necrotic-type hybrids was continued. In all 3 generations after the second backcross, approximate 1:1 ratios between plants of necrotic and mottling types were obtained. A detailed account of these 5 backcross generations is given in table 1.

Self-fertility comparable to that of the original parents, not observed in earlier hybrid generations, appeared in the third backcross generation. A necrotic-type plant of this generation was selfed. Altogether 288 plants were grown from its seed and tested; the ratio of necrotic-type to mottling-type plants was 210:78, or slightly less than a 3:1 ratio (Table 2, lot 1).

TABLE 2.—*Ratios of necrotic-type to mottling-type plants in 2 selfed generations of Nicotiana paniculata-rustica hybrids*

Lot number	Parent (selfed)	Necrotic-type plants	Mottling-type plants	Totals	Observed ratios
1	Necrotic-type plant of third backcross generation	210	78	210:78	2.92:1.08
2	Necrotic-type plant of lot 1	42	14	172:57	3.01:1.00
3	" " " " " "	45	16		
4	" " " " " "	35	7		
5	" " " " " "	50	20		
6	" " " " " "	67	0	128:0	1:0
7	" " " " " "	61	0		
8	Mottling-type plant of lot 1	0	59	0:157	0:1
9	" " " " " "	0	98		

Eight lots of plants were grown from seeds obtained by selfing 6 necrotic-type and 2 mottling-type plants of this generation. Four of these populations, grown from seeds of necrotic-type parents, showed approximate 3:1 ratios between necrotic-type and mottling-type plants (Table 2, lots 2 to 5). The other 2 populations derived from necrotic-type parents contained only necrotic-type plants, 128 in all (Table 2, lots 6 and 7); these resembled *Nicotiana paniculata* in appearance and were self-fertile. The 2 populations derived from mottling-type parents contained only mottling-type plants, 157 in all (Table 2, lots 8 and 9).

These results indicate that a single dominant gene *N* (necrosis), derived from *Nicotiana rustica*, determines a necrotic type of response to infection with tobacco-mosaic virus, its absence allowing mottling. It is not known just how the *N. rustica* gene *N* finally associated itself with the chromosomes of *N. paniculata*, but it seemed to be able to act in a uniform manner in the twice self-pollinated sets, as it had in backcross populations.

The production of 2 sets containing only necrotic-type individuals resembling *Nicotiana paniculata* in appearance constitutes evidence that the

necrotic-type response of *N. rustica* was incorporated successfully in *N. paniculata*-like segregates in homozygous condition. This was confirmed by reciprocal crosses of one of these individuals with mottling-type *N. paniculata* (*nn*). Only necrotic-type (*Nn*) plants were obtained, 174 with mottling-type *N. paniculata* as pollen parent, and 204 with mottling-type *N. paniculata* as seed parent. It is evident that the gene *N* is capable of being distributed regularly to gametes of both sexes.

Throughout the study of backcross sets only one kind of necrotic-type response to inoculation with tobacco-mosaic virus had been noted. It was not realized that this differed slightly from the response of *Nicotiana rustica*, until certain sets produced by selfing showed two kinds of necrosis. The mode of occurrence of these gave evidence of the presence of a modifying gene.

Five of 8 sets, derived by self-pollination from necrotic-type plants of a fourth backcross set, each showed two kinds of necrotic-type response. One of these was like that shown by all plants in backcross progenies, involving unmodified necrosis. The other was a delayed necrotic-type response associated with peripheral yellowing of lesions. In plants showing the latter response, both primary and secondary lesions were yellowish, with necrosis varying from a trace to a considerable amount; in the systemic phase of the disease this yellowing superficially resembled ordinary mottling. *Nicotiana rustica* responds to infection with necrotic lesions, each of which is surrounded by yellowed tissue. These lesions tend to appear later than those of the necrotic-type backcross plants. In the 5 sets of plants showing the delayed necrotic-type response (Table 3, lots 1-5), the ratio of unmodified necrosis to delayed necrosis was 100:33. This 3:1 ratio appears to indicate that delay of necrosis, with attendant yellowing of surrounding

TABLE 3.—Numbers of plants showing 3 types of disease response, unmodified necrosis, delayed necrosis, and mottling, in 8 lots grown from seeds obtained by selfing 8 necrotic-type plants of the fourth backcross generation (see Table 1)

Lot number	Types of disease response		
	Unmodified necrosis	Delayed necrosis	Mottling
1	20	7	17
2	6	1	4
3	24	5	27
4	24	7	32
5	26	13	26
6	28	0	27
7	43	0	26
8	31	0	33
Totals	235		192

tissue, is dependent on a recessive gene (*d*, delayed necrosis). The recessive gene was evidently not derived from *N. paniculata*, since delayed necrosis was not observed in 5 preceding hybrid generations, representing *N. paniculata* crosses. It must, therefore, have come from *N. rustica*. Furthermore, this gene was not in the chromosome bearing the gene for necrosis (*N*), for the two showed independent segregation. Thus, there was evidence of persistence of a chromosome or chromosome fragment from *N. rustica*, not intentionally selected, as was the carrier of the dominant factor *N*, but probably substituted for a *N. paniculata* chromosome from which it differed by at least one gene. The remaining 3 selfed sets from necrotic-type plants of the fourth backcross set showed no delayed necrotic-type response. Two of them (Table 3, lots 6 and 8) had earlier shared a common parent, a necrotic-type plant of the third backcross generation, with the 5 sets that did show delayed necrosis; the remaining set (Table 3, lot 7) was also closely related.

Simple genetic ratios were observed in all backcross sets, with the possible exception of the second backcross generation as noted; the selfed generations summarized in table 2 also showed customary ratios. In table 3, however, it will be noted that there was a consistent excess of mottling-type plants over the expected 3:1 ratio in all selfed sets derived from plants of the fourth backcross generation. Evidence in hand does not seem sufficient to warrant a conclusion as to the reason for this. Segregation of genes *N* and *D* in these sets seemed regular.

Some evidence was obtained confirming the relationship of *Nicotiana rustica* to other species. It is believed that *N. rustica* ($n=24$) originated as an amphidiploid from a cross involving progenitors of *N. undulata* ($n=12$) Ruiz and Pavon and of *N. paniculata* ($n=12$) (1, pp. 393-395). The response of *N. undulata* to inoculation with tobacco-mosaic virus has not been tested previously. If this explanation of relationships is correct, however, it should resemble the necrotic type displayed in *N. paniculata-rustica* hybrids, unless present-day stocks of *N. undulata* or of *N. paniculata* have lost a dominant gene *N*, now characteristic of *N. rustica*. Seeds of *N. undulata* were obtained through the kindness of Dr. R. E. Clausen, of the University of California. Plants grown from 2 seed lots responded to inoculation with tobacco-mosaic virus by systemic necrosis of the type shown otherwise only by *N. rustica* and the *N. paniculata* hybrids described in this paper. Their response appears to confirm the relationship of the species. Plants from a third lot of seeds of *N. undulata* gave an atypical mottling-type response, which may indicate either a lack of the gene *N*, or another modification of the necrotic-type response similar to that in hybrids of constitution *NNdd* or *Nndd* as described above. The true nature of this response cannot be decided in the absence of genetic data. From the same source 2

other previously untested *Nicotiana* species were obtained: they were *N. solanifolia* Walp. and *N. raimondii* MacBride, both morphologically distinct from, but genetically closely allied to *N. paniculata*; they both showed a mottling-type response to inoculation with tobacco-mosaic virus, similar to that of *N. paniculata*.

DISCUSSION

A number of hosts of tobacco-mosaic virus respond to infection by simultaneous primary necrosis and localization of virus. This is true, for example, of *Nicotiana glutinosa* L., *N. langsdorffii* Weinm., and *Phaseolus vulgaris* L., which have been used commonly for estimating virus concentration and separating virus strains. The view has recently been expressed by some, that there is a causal relationship between necrosis as a disease symptom and localization of virus. In mature or nearly mature plants of *Nicandra physalodes* (L.) Pers., however, infection with tobacco-mosaic virus is followed by the production of non-necrotic local lesions only. These are marked by starch retention, slight yellowing, or, in old leaves, chlorophyll retention (3, pp. 344-345). Jensen (6) has described yellow-mottling strains of tobacco-mosaic virus that produce only nonnecrotic primary lesions in *Nicotiana tabacum* L. and do not spread systemically. Thus it would appear that localization of virus may occur without necrosis. Systemic necrosis is a comparatively infrequent sequel of infection with tobacco-mosaic virus. It has been described for several species, however, including *N. rustica* (3, pp. 333-337). The present demonstration of a purely necrotic-type systemic disease, with no trace of mottling, in which virus movement is as prompt as in the corresponding mottling-type disease in similar young plants of but slightly different genetic constitution, furnishes an ideal instance of necrosis without virus localization. Since virus localization may occur without necrosis, and necrosis without localization, it appears that there is no necessary causal interrelationship between necrotic-type response and localization of virus.

It is not yet clear what mechanism causes necrotic-type response in the cells of plants receiving the dominant genetic factor *N* (necrosis) to replace the mottling-type response, characteristic of plants of *Nicotiana paniculata* lacking this factor. Evidently, however, it is a mechanism not affected by the numerous chromosomal recombinations involved in the second, third, and fourth hybrid generations described here, in which plant types were found to be extremely varied, but in which only these 2 types of disease response were noted.

Hybrids were made to approach *Nicotiana paniculata* in appearance by means of repeated backcrosses. The dominant gene *N* derived from *N. rustica* was retained as a result of simultaneous selection, and was finally

established in homozygous condition by self pollination of necrotic-type plants. Since the plants are now homozygous for the introduced genetic factor, are self-fertile, and would be identified as *N. paniculata* if found growing wild, the transfer of the *N. rustica* gene is considered an interspecific transfer, and the new strain may for practical purposes be considered a necrotic-type variety of the species *N. paniculata*. It may, of course, differ from the original *N. paniculata* stock in ways not now recognized, for it is probable that not a single gene, but a segment of a chromosome, was carried over from *N. rustica*.

SUMMARY

A necrotic type of response to infection with tobacco-mosaic virus was introduced into the species *Nicotiana paniculata*. This was accomplished by transferring a dominant gene *N* (necrosis) from *N. rustica*, through repeated backcrosses of the hybrid *N. paniculata* × *N. rustica*, using *N. paniculata* pollen, but retaining in each generation only individuals responding to inoculation by production of necrotic lesions. The necrotic-type variety of *N. paniculata* thus produced was self fertile and, in appearance, resembled the ordinary mottling-type *N. paniculata*. In its response to infection, however, it was essentially like *N. rustica*, dying from systemic necrosis if infected when young, localizing virus if infected when old.

A dominant gene *D* (unmodified necrosis), not found in *Nicotiana rustica*, was observed in the newly derived necrotic-type *N. paniculata* plants. It was found to segregate independently with respect to the gene *N* (necrosis). In the presence of *N*, the gene *D* allowed necrotic primary lesions to appear promptly, and prevented extensive yellowing of surrounding tissue.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

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BLACK STEM OF ALFALFA IN IDAHO¹

RUTH REMSBERG AND C. W. HUNGERFORD

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INTRODUCTION

A disease of alfalfa, which causes a distinct blackening of stems and petioles and a spotting of the leaves, has been especially prevalent in Idaho during the last 2 years. In previous years various collections and observations of discolored alfalfa stems have been made. The cause usually was attributed to *Phytomonas medicaginis* Sack., the organism causing bacterial stem blight. Careful culture work, involving numerous collections during 1934 and 1935, revealed the fact that a fungus organism is, in the majority of cases, associated with dark brown or black lesions so often found on alfalfa stems in Idaho. Valteau and Fergus (5), and later Johnson and Valteau (1), reported a fungus disease on alfalfa in Kentucky, which they have named black stem. They state that black stem is caused by *Phoma medicaginis* Malbr. and Roum. From their description of the disease and the causal organism, it appears that the disease with which we have been working in Idaho is the same as the one occurring in Kentucky.

Richards (2) recently reported upon resistance of certain alfalfa varieties to stem blight in Utah. Although he intimates that the disease under discussion is the bacterial stem blight (*Phytomonas medicaginis*) he states in a footnote that a species of *Phoma* has been isolated with considerable uniformity. Richards did not describe the symptoms of the disease in question.

OCCURRENCE OF BLACK STEM IN IDAHO

Following the mild winter of 1933-1934, an outbreak of stem rot of alfalfa and clover, caused by *Sclerotinia trifoliorum* Eriks., occurred in certain sections of northern Idaho. The stems of stem-rot diseased plants submitted for examination were badly blackened in many cases, and cultures from these lesions indicated the presence of a species of *Phoma*. A careful study has been made of this type of injury upon alfalfa, and the black-stem disease has since been collected on alfalfa from 12 different, widely distributed counties in Idaho. It appears that the disease is generally distributed throughout the State. The bacterial stem blight described by Sackett (4) is also widely distributed in the State, as evidenced by observations made

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during the last 20 years. The early symptoms of the bacterial disease differ sufficiently from black stem to make it easily possible to differentiate between them. Later in the progress of the bacterial disease, lesions turn darker and more closely resemble the lesions of the black-stem disease.

The damage caused by black stem is much more severe on the first cutting of alfalfa. As a matter of fact, it is rather difficult to find the disease on later cuttings. It is to be found throughout the season, however, on stubble and on plants that have not been cut for hay. Although the percentage of loss and reduction of stand from this disease is difficult to estimate, observations would indicate that this loss has been considerable where the disease has been severe.

DESCRIPTION OF THE DISEASE

The characteristic symptoms of the disease consist of dark brown to black areas on the stems and petioles, and black spots on the leaves (Fig. 1, A). Under field conditions in Idaho, the blackened areas are largely confined to the stems and petioles, with few leaf spots, while under greenhouse conditions, the leaf spots are more conspicuous. The stem lesions originate as small black spots, which rapidly enlarge, coalesce, and form larger areas. These areas may extend for several inches along the stem and may entirely girdle it. In the early stages of the disease the infection is confined mainly to the surface of the stem, but later may penetrate to the interior. Leaves that are severely spotted become etiolated and finally drop off. In heavy stands of alfalfa, young shoots may be injured and die, or the tips may be killed back. This is especially true under nonirrigated conditions when the spring season is long, wet, and cool, and also in irrigated regions where conditions are favorable for rank growth and lodging of plants. Under such conditions stems may be blackened their entire length and become badly distorted and dwarfed. On dead stems and leaves, fruiting bodies of the organism are present in abundance (Fig. 1, C).

THE CAUSAL ORGANISM

The causal organism is easily isolated from blackened stems and petioles and from the spots on the leaves. It also is possible to isolate the same organism from the fruiting bodies on the dead tissue. The organism was isolated in March, 1935, from black areas on dead alfalfa stems collected and kept in herbarium folders since 1925.

A culture of *Phoma medicaginis*, obtained from W. D. Valleau in Kentucky, proved to be identical with the organism isolated from alfalfa in Idaho. Several cultures, isolated from alfalfa collected in various parts of Idaho, and the one from Kentucky were compared on potato dextrose, cornmeal, prune, and alfalfa-extract agar, and on sweet-clover stems, when

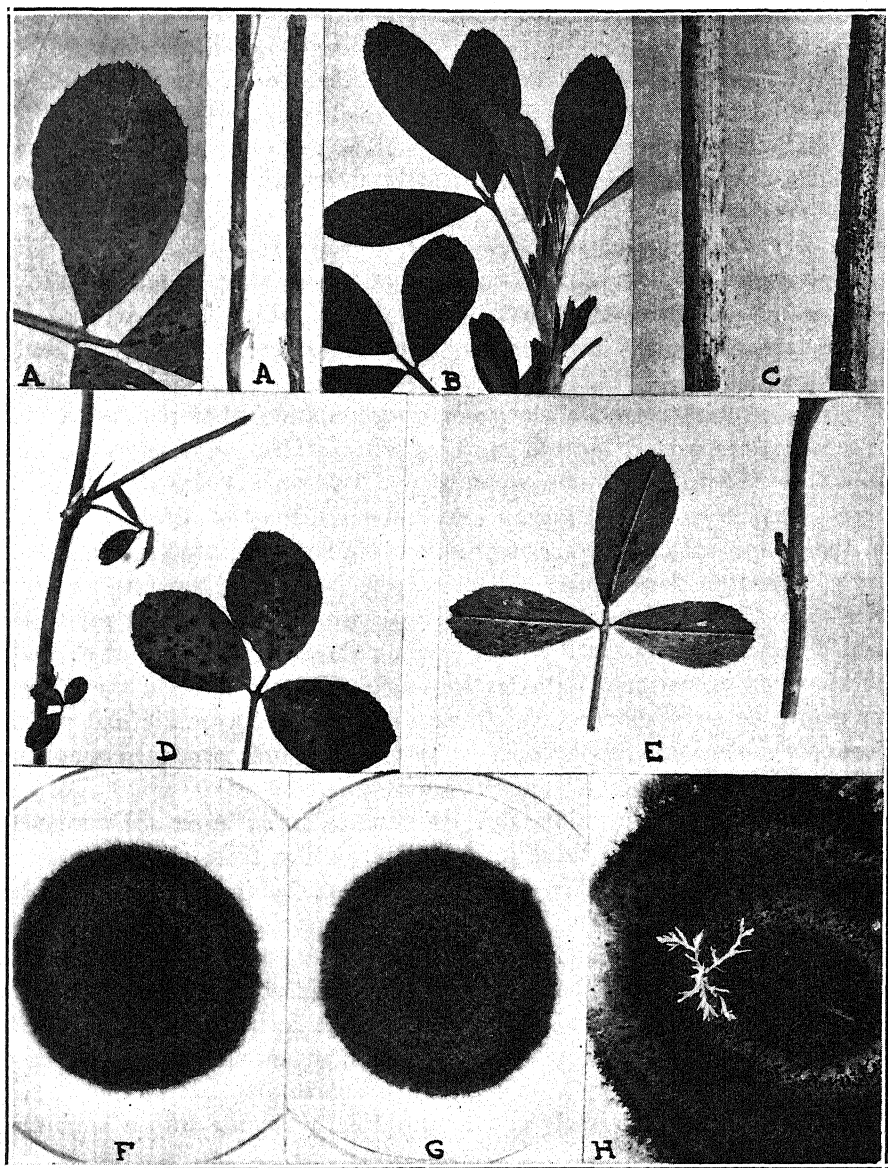


FIG. 1. A. Symptoms of black stem on alfalfa leaves and stems from the field. B. Healthy alfalfa. C. Fruiting bodies overwintering on alfalfa stems. D. Stems and leaves from alfalfa plant, inoculated with *Phoma medicaginis*. E. Stems and leaves from plant, inoculated with *Pleospora rehmiana*. F. Culture *P. medicaginis*, reisolated from inoculation (D). G. Culture of *P. medicaginis*, reisolated from inoculation with *Pleospora rehmiana* (E). H. Typical crystals that appear in cultures of *P. medicaginis*.

grown under different conditions of light and temperature. They were compared at room temperature under normal light and darkness, at 0°–5° C. in total darkness, and at 26° C. in total darkness. All these cultures appeared identical in reaction to media and in growth characteristics. Pycnidia appeared in four to six days. These pycnidia, at first, were soft and light brown, but became hard and black, when mature. The pycnosporos escape from the ostiole in a gelatinous mass or ribbon, and are cylindrical or oblong, hyaline and unicellular. However, many 2-celled pycnosporos have been found in older cultures. Crystals appeared in all potato-dextrose agar cultures in 4 to 10 days (Fig. 1, H). These crystals are typical formations of *P. medicaginis* on potato agar and also were noted by Johnson and Valteau (1).

It was noticed, on close microscopic examination, that perithecia of a *Pleospora* were present on old dead tissue of alfalfa, especially the dead stems from the previous year. Johnson and Valteau (1) also report having observed a *Pleospora* fruiting on overwintering stems of alfalfa. Similar perithecia appeared on sweet-clover stems that had been used in the laboratory as a medium for culturing *Phoma medicaginis*, after 3 month's growth at 0°–5° C. Single-spore isolations of ascospores from these perithecia yielded cultures somewhat different from single spore isolations of *P. medicaginis* when grown on potato-dextrose agar. However, when the culture was grown on sweet-clover stems, it produced typical pycnidia and pycnosporos. Single-spore reisolations of these pycnosporos produced a culture identical to that of the original *P. medicaginis*.

In brief, then, the pycnidia and pycnosporos found in nature were comparable in every way to those produced in culture from tissue isolations. The ascigerous stage found in nature was the same as that developed in cultures of the imperfect form, and ascospore cultures from nature and from cultural media produced the imperfect stage of the fungus.

It appears, then, that the *Pleospora* is the perfect form of *Phoma medicaginis*, and it has been identified as *Pleospora rehmana* (Staritz) Sacc. (3, p. 1033). The perithecia are immersed to somewhat free; the ascus is elongate-clavate, 115–130 × 28–35 μ ; the ascospores are in 2 to many rows, yellowish brown, transversely and longitudinally septate into a muriform condition, 30–35 × 15–18 μ .

PATHOGENICITY

Plants were successfully inoculated in the greenhouse and laboratory by different methods. In some cases, inoculation was made by transferring the organism on agar directly from cultures to the healthy tissue. In other cases, plants were sprayed with a suspension of pycnosporos of the causal

organism. Infection in all cases was found to occur readily, even when the plants were not wounded when inoculated.

Alfalfa plants, brought into the greenhouse from the field, Grimm and common alfalfa, and yellow-blossom sweet clover plants that had been grown from seed in the greenhouse were successfully inoculated with pure cultures of the organism. Typical symptoms of black stem were observed 2 to 4 weeks after inoculation. Black lesions appeared on stems and petioles and numerous black spots developed on the leaves (Fig. 1, D). The causal organism, *Phoma medicaginis*, was easily reisolated in all cases (Fig. 1, F).

Common and Grimm varieties of alfalfa and yellow-blossom sweet clover plants also were inoculated in the greenhouse with cultures of *Pleospora rehmiana*. Symptoms were produced that were identical to those produced by *Phoma medicaginis* (Fig. 1, E). In this case, also, the organism that was reisolated was *Phoma medicaginis* (Fig. 1, G).

Single-spore cultures of *Pleospora rehmiana* and single-spore cultures of *Phoma medicaginis* produced the same symptoms on alfalfa and sweet clover stems and leaves in moist chambers in the laboratory. Spotted areas on stems, petioles, and leaves appeared in 2 to 5 days, and pycnosporos were produced in 5 to 8 days after inoculation.

SUMMARY

The black-stem disease of alfalfa in Idaho appears to be the same disease as that reported from Kentucky. The causal organism is identical with *Phoma medicaginis*, which has been identified by Valleeau as causing black stem of alfalfa in Kentucky. *Phoma medicaginis*, when grown on sweet clover stems, produced an ascigerous stage corresponding to *Pleospora rehmiana*, which was found also on dead alfalfa stems from the field. Pycnidia of *Phoma medicaginis* were produced on alfalfa and sweet clover in moist chambers after inoculation with a culture of *Pleospora rehmiana*. Typical black-stem symptoms were produced also on alfalfa and sweet clover in the greenhouse when inoculated with *Pleospora rehmiana*; when reisolated, it proved to be identical with the original culture of *Phoma medicaginis*.

It is demonstrated that *Pleospora rehmiana* is the perfect stage of *Phoma medicaginis*, the ascospores of which also function as inoculum for black stem in the alfalfa fields in the spring.

DEPARTMENT OF PLANT PATHOLOGY,
COLLEGE OF AGRICULTURE,
UNIVERSITY OF IDAHO.

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THE DISSEMINATION OF SEPTORIA ACICOLA AND THE EFFECT OF GRASS FIRES ON IT IN PINE NEEDLES¹

A. F. VERRALL

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INTRODUCTION

Septoria acicola (Thüm.) Sacc. causes a serious needle blight of longleaf pine seedlings. Investigations of the effectiveness of periodic grass fires in controlling the disease in natural reproduction² have led those working with it to believe that its dissemination is chiefly by spores carried in rain drops, splashing from infected plants. The present investigations were made in Washington Parish, Louisiana, in 1933, to get more precise information on the dissemination of the fungus and the effect of grass fires on it in needle tissue.

THE DISSEMINATION OF SPORES

The general infection of the 1932 needles to a height of 6 feet by *Septoria acicola* in the Bogalusa area of Washington Parish and the presence of a noticeable amount of the bar-spot type of infection (1 to 2 per cent of the leaf length dead on some trees)³ on mature trees at a height of 75 feet indicates that, under certain conditions, *Septoria* can be appreciably wind-disseminated. There was indirect evidence, however, that during the first half of 1933 wind dissemination was not abundant. Seedlings over 2 feet high did not develop many spots on the 1933 foliage, although on smaller seedlings infection was of epidemic rating as usual.

There also exists the possibility of dissemination by insects, especially by mites and a large grasshopper common during the winter months and early spring. However, no spores were found by direct microscopic observation or in washings of insects. The culture method would be better for such studies, but *Septoria* does not lend itself to this method, and direct observation of spores on opaque objects is not always easy or certain. However, if insects commonly carried large numbers of spores, some probably would have been found.

¹ The investigations here reported were made by the writer while employed as Field Aid by the Division of Forest Pathology, Bureau of Plant Industry, in cooperation with the Southern Forest Experiment Station, U. S. Forest Service, New Orleans, La. Much assistance was given by Mr. P. V. Siggers of the Division of Forest Pathology.

² Siggers, P. V. Observations on the influence of fire on the brown-spot needle blight of longleaf pine seedlings. Jour. Forestry 32: 556-562. 1934.

³ The bar-spot type of infection is indicative of resistance. On such spots the fungus seldom fruits or develops extensively. (Verrall, A. F. The resistance of saplings and certain seedlings of *Pinus palustris* to *Septoria acicola*. Phytopath. 24: 1262-1264. 1934).

Direct evidence tended to show that most of the spores are disseminated by splattering rain. From the middle of January until June 30, 1933, trap slides were kept in an epidemic area of dense seedling stocking, and examined daily. Standard microscope slides were smeared with vaseline as an adhesive until June, when the temperature became high enough to liquefy vaseline. A thin layer of "Tree Tanglefoot" was then used. Slides were placed horizontally and vertically sufficiently near diseased needles to be splattered by rain drops hitting the leaves and at heights of $1\frac{1}{2}$ and 3 feet, respectively, above seedlings. During the 6 months, numerous spores were caught on 3 occasions: January 24-25, May 1-2, and June 10-11. On these occasions and on at least one day previous, it rained, so that there were at least 29 hours with relative humidities above 80 per cent each time spores were caught. Slides placed near enough to diseased seedlings to be splattered by rain drops from infected seedlings collected 100-400 spores per slide, and on May 1 and 2 when heavy winds accompanied the rain 50 spores per slide were caught at 3 feet above seedlings. During the shorter rains on other days a few spores were caught under diseased plants, but, between rains, only 3 solitary spores were caught. The duration of the wet period in the usual summer shower often is not long enough for spore discharge, the fruiting bodies presumably drying before spores are discharged. The temperature probably is too low for sporulation during many winter rains; the January 24-25 discharge was, however, an exception, the weather at that time being warm ($53-80^{\circ}$ F. on January 23 to 25).

The theory that spore dissemination occurs mainly through the splashing of rain also was tested by dividing a 10×10 foot plot into 2 sections. On one the infected 1932 foliage was left on the plants; on the other, all 1932 foliage was removed and the section screened on the sides with a double thickness of cotton cloth 15 inches high. Within 5 to 10 feet of the plot were numerous seedlings with heavily infected 1932 foliage extending more than 15 inches above the ground so that it is likely that any wind-blown spores from these could reach the plants within the screened plot. All the seedlings over 12 inches high were removed from a 5 foot border around the plot to prevent splattering over the screen. The new foliage on the plants in the nonscreened part of the plot were subject to inoculation by spores from infected 1932 foliage, both within and surrounding the plot, as well as by any wind-disseminated spores. At the time of establishment in March, 1933, some of the small seedlings had leaf buds opening, but the buds of most of the plants had not yet opened. On May 20, there was a noticeable difference in the degree of infection on the two plots, the new foliage on the plants within the screen being fairly free (3.9 per cent of the leaf length dead), while those without were heavily infected (14.4 per cent of the leaf length dead). On June 23 the amount of the 1933 leaf length dead was 7.6 and

25.0 per cent, respectively. This infection appeared to be due chiefly to the spore shower of May 1-2.

If the spores of *Septoria* were commonly wind-disseminated one would not expect much decrease in the degree of infection on fire lanes 100 feet wide in an epidemic area as compared with the adjacent unburned areas. Fire lanes are made in the fall by burning strips about 100 feet wide at strategic places across the area to be protected. The grass and the needles of the previous year on pine seedlings are burned. The lanes are flanked by a narrow plowed zone. In the Bogalusa area, however, there was a marked reduction in the infection on fire lanes (Table 1). Each figure is based on 50 seedlings. Only the 1933 needles were included in the estimate.

TABLE 1.—Percentage of leaf length dead on June 20, 1933, on adjacent burned and unburned areas in Washington Parish, Louisiana

Lane no.	Unburned to right of lane	Right 5 ft. of lane	Center of lane	Left 5 ft of lane	Unburned to left of lane
1	15.3	3.6	2.1	4.2	15.2
2	15.4	4.8	1.6	2.9	15.1

A 15-foot road borders the right edge of lane 1 and about 5 feet of plowed soil on the opposite side and both edges of lane 2. Similar reductions in the degree of infection have been observed on other fire lanes.

The behavior of the disease on burned areas⁴ suggests that there is but little wind dissemination. The fire, if severe, destroys most of the old infected leaves, and the wind brings in a few spores that cause a low degree of infection the first season. During the spring of the second year, with the rapid extension of the fungus from the points of inoculation, enough spores are produced for a general inoculation of the new foliage through splattering of rain, resulting in the usual great increase in the amount of infection the second season after a burn in areas of severe seedling infection, even though it takes 5 or 6 growing seasons after a burn for infection to reach a maximum.⁴

THE EFFECT OF FIRE OF SEPTORIA IN PINE NEEDLES

The severity of grass fires varies greatly, depending on a number of factors; and the amount of longleaf seedling needles killed by the fire likewise varies from a browning of the tips to complete destruction. A number of moist chamber and agar cultures were made of leaves to determine what fire intensities sterilize brown-spotted needles. The 6 fires examined occurred in January, February, March, and April, 1933, and in no case did

⁴ Siggers, P. V., *loc. cit.*

Septoria fruit, nor could isolations be obtained from leaf tissue killed by fire. The killed tissue used varied from charred to that uncharred and apparently killed by temperature near the thermal death point of leaf tissue. Plantings were made within 2 days of the fire and again 1 to 3 weeks later. In all, 96 plantings were made on agar and about 500 moist chamber needles were examined without finding living Septoria. Plantings, just after a fire, were all sterile; those after a week yielded mostly Pestalozzia.

Fruiting was abundant on unburned leaves in the same area at the time of these tests and cultures were easily secured from them. Fruiting also was abundant on the green bases of needles with fire-killed tips up to the end of the live portion and also for a period up to 2 weeks on the dead areas gradually dying back from the fire-killed tips. Pestalozzia, Lophodermium, and other fungi soon take over the fire-killed tips and, as the green bases die, quickly exclude Septoria.

SUMMARY

The indirect and direct evidence available indicates that the spores of *Septoria acicola* are wind-disseminated to a slight extent, and that most dissemination is local by spores carried in rain drops splattering from diseased plants. It appears that for abundant spore discharge 2 or more warm rainy days are needed. Ordinary summer showers are not of sufficient duration for much spore discharge and during winter rains the temperature appears to be too low for spore discharge.

Temperatures that kill leaf tissue also kill the Septoria in that tissue.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

A DISPOROUS GNOMONIA ON PECAN

J. B. DEMAREE AND J. R. COLE

(Accepted for publication December 7, 1935)

In 1917 Matz¹ reported the occurrence in Florida of a *Gnomonia* on pecan leaves in the ascus of which only two spores were produced. The following year he again referred to the disease as pecan leaf-blotch caused by *Gnomonia* sp. Apparently, there is no record of later collections of the fungus until the writers collected it in the summer of 1928 in a pecan orchard near Monticello, Florida. The following year it was found again in the same orchard, and also at Thomasville, Putney, and Leesburg, Georgia. Since then it has frequently been observed in various orchards in southern Georgia.

Specimens of the fungus collected by Matz on pecan leaves in 1916 were preserved by the Office of Pathological Collections, United States Department of Agriculture, and are listed as *Gnomonia* sp. Those specimens have been examined by the writers, and, from all appearances, the fungus is the same as the one here described.

THE DISEASE

About the middle of June lesions first appear on the leaves as small, inconspicuous brown spots, showing on both sides of the leaflet, with no certain diagnostic features. The spots enlarge rather rapidly and frequently attain a diameter of 0.75 inch. Normally the necrotic areas are circular, but their spread is often so limited by the midrib and larger leaf veins that they become long and narrow. The spots frequently occupy 2 and perhaps 3 adjacent spaces formed by the leaf veins. (Fig. 1.)

The spots vary from a light brown or tan to almost black. The shade of tan is associated with the production of perithecia, which appear in mid or late July. Were it not for the perithecial beaks and the characteristic delimitation of the spots by the vascular tissues, the disease might be confused with lesions caused by *Cercospora fusca* Rand. The number of initial infections on leaflets is commonly not large, varying usually from 1 to 5, but the spots became numerous after secondary infections take place.

THE FUNGUS

No conidial stage has been found on either affected leaflets or artificial cultures. The surfaces of the lesions on both sides of the leaflets are devoid of hyphae, except that a scant amount of white mycelium may be present along the advancing edge of the young spots. These aerial hyphae, however,

¹ Matz, J. An undescribed *Gnomonia* on pecan leaves. Florida Agr. Expt. Sta. Ann. Rept. 1917: 89R-94R. 1918.

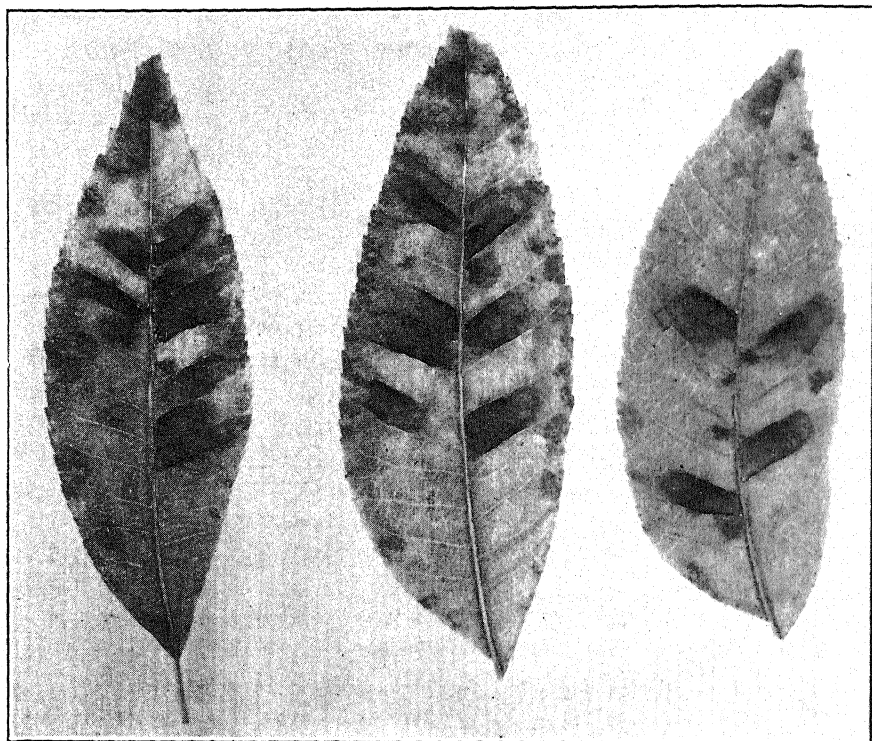


FIG. 1. Pecan leaflets spotted with *Gnomonia dispersa* n. sp.

are found only during extended periods of frequent rains and overcast skies, or when affected leaflets are kept in a moist chamber.

The perithecia form and mature on the living leaves in late summer. Mature ascospores have been found as early as mid-July, only 4 or 5 weeks after the first evidence of infections.

The perithecia are buried within the spongy mesophyll of the leaf tissues and not infrequently cause the upper epidermis of the leaf to bulge and even break. The ostioles or beaks are black and frequently curved; most of them penetrate the lower or ventral side of the leaf surface. Only rarely do they break through the upper epidermis.

The perithecia, exclusive of the beak, are spherical to ellipsoidal with thin walls of only 2 to 4 cells thickness. The walls of the beak are thicker and composed of smaller cells than those of the main body of the perithecium.

Two spores commonly are formed in each ascus, and occasionally there is but 1, and less often 3 or 4. The ascus wall is hyaline, very thin, and frequently seems to be distended by the spores. (Fig. 2.) The base of the ascus is drawn out into a needle-shape appendage, which, when detached,

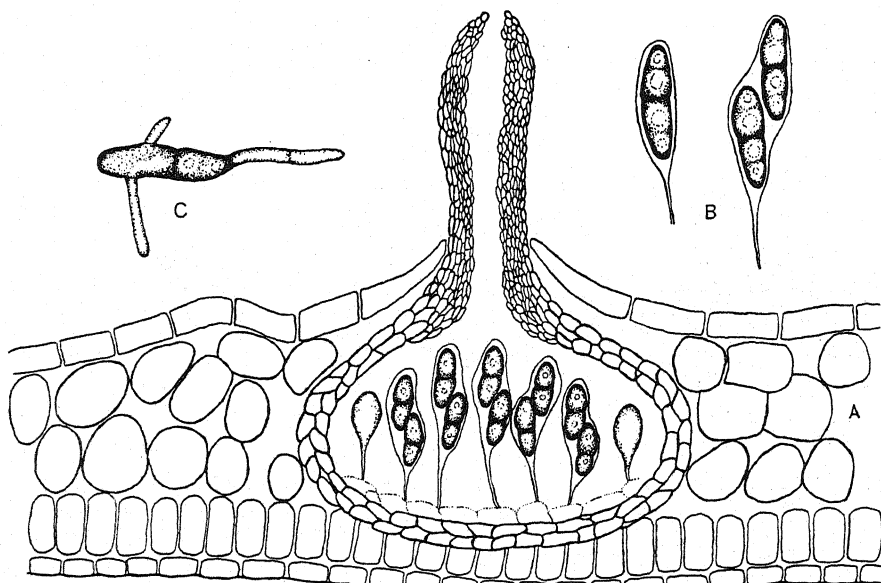


FIG. 2. *Gnomonia dispersa* n. sp. A. Perithecium ($\times 500$); B. Asci ($\times 625$); C. Germinating spore ($\times 650$).

often is bent downward in the form of a hook. The shape of the asci varies and seems to be determined by the arrangement and number of the spores. They are cylindrical when a single spore is present or when two spores lie in a row; ovoid to obovate when the spores are side by side; and irregularly spindle-shape when the spores are imbricate.

The spores are guttulate, 1-septate, hyaline, slightly constricted at the septum, and vary considerably in size. When a single spore is formed in an ascus, it is much larger than when 2, 3, or 4 are present, but there may be 1 large and 1 small spore in the same ascus. When 3 spores are present in a single ascus, 2 may be about normal size and 1 smaller, or there may be 2 small spores and 1 large one. A very few 4-spore asci have been observed and they contained only small spores. The cells of the spores are of unequal size; but this character is also variable, as the difference may be only slight. When an ascus contains 2 spores, the smaller cells usually are situated at opposite ends of the ascus. The germ tubes may project from the sides or from either end of the spores.

Apparently, Matz did not consider the fungus of sufficient importance to furnish measurement, and did not propose a specific name. The fungus is found quite frequently in southern Georgia and northern Florida on pecan and on other species of *Hicoria*. The disease is considered only a minor one on pecans, and is often associated with rosette, a zinc-deficiency disease.

Since other species of the genus *Gnomonia* have been described as occurring on overwintered leaves of the hickory group of trees, 2 being the perfect stage of forms causing serious disease of pecans, it has been found inconvenient to refer to this 2-spore form in literature as *Gnomonia* sp. To clarify the matter, it is proposed here to give this 2-spore form the name *Gnomonia dispersa*.

As far as the writers can learn, there has been no disporous *Gnomonia*, or in fact, any disporous ascomycete, with the possible exception of *Microsphaeria alni* (Wallr.) Wint., described as causing lesions on green leaves of the *Hicoria* group of plants.

This *Gnomonia* on the pecan differs widely in several respects from other species of the genus described on *Hicoria*. These differences may be summarized as follows: This *Gnomonia* commonly has 2, occasionally 1, and rarely 3 or 4 spores in each ascus. The fungus causes a characteristic spotting on the living leaves and the perithecia develop and mature during the latter half of the season, while the leaves are still on the trees. No conidial stage has been found associated with this fungus.

All other forms of *Gnomonia* described on *Hicoria* have been reported to have 8 spores in each ascus, and their perithecia develop only on overwintered leaves. The writers believe that the disporous form is an undescribed species with the following description: *Gnomonia dispersa*, n. sp.

Perithecia scattered, submerged, subglobose to flattened, submembranaceous, dark-brown to black, 80–150 μ by 100–160 μ , neck long, protruding, black. Asci cylindrical, irregular, thin-wall, 2-spore, 55–77 μ by 14–17 μ . Paraphyses none. Spores uniseptate, slightly constricted at septum, spindle-shape to cylindrical, hyaline, guttulate, 24–51 μ by 5–10½ μ .

Habitat: On living leaves of *Hicoria pecan* Britt., Monticello, Fla.; Thomasville, DeWitt, and Leesburg, Ga. Type specimens have been deposited in the Herbarium of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Pure cultures of *Gnomonia dispersa* were obtained by first macerating the perithecia, then picking the ascospores out with a needle with the aid of the binocular microscope and placing them in sterile Petri dishes of cornmeal and lima-bean agar. Germination took place within 24 hours at room temperature. Germinated spores were then transferred to Petri dishes and tubes of agar. The fungus was grown on a variety of artificial media, but made the best growth on a medium of cornmeal and potato agar. It grew rapidly on this substratum and attained a colony diameter of approximately 5 cm. in 30 days. Perithecia formed in abundance in about 20-day-old cultures of cornmeal-potato agar when kept near the optimum temperature of 24° to 26.5° C. The perithecia, asci, and ascospores developed in cultures were in no way different from those produced on the host under natural conditions. No conidia were observed in any of the cultures on artificial media.

Cornmeal-agar cultures with a pH range of 3.8 to 9.5 were kept at the optimum temperature over a period of 30 days. These studies also indicated that the organism thrives best on an acid or slightly alkaline medium. The maximum colony growth, 5 cm. in diameter, was made on media ranging on the pH scale from 5.0 to 6.5. On more alkaline agar the fungus grew very slowly.

SUMMARY

A spotting of pecan foliage caused by a fungus, previously reported by Matz, is described. Matz did not suggest a specific name, but referred to it as *Gnomonia* sp., and it is here described as *Gnomonia dispora* n. sp.

Perithecia form and mature in necrotic areas on the living leaves in late summer.

Commonly 2 spores, occasionally 1, and less often 3 or 4, are formed within a single ascus. As a rule, when there is only a single spore in an ascus it is abnormally large; when there are 2 per ascus they usually are about coequal; and when there are 3 one of them may be abnormally large and the other 2 small, or there may be 2 average-size spores and 1 much smaller.

No conidial stage has yet been found.

Normal perithecia, asci, and spores were grown on a mixture of cornmeal and potato agar in about 20 days at the optimum temperature of 24° to 26° C.

ALBANY, GEORGIA

CONCENTRATION OF AMMONIA NECESSARY IN A LOW-LIME PHASE OF HOUSTON CLAY SOIL TO KILL THE COTTON ROOT-ROT FUNGUS, *PHYMATO- TRICHUM OMNIVORUM*

D. C. NEAL AND EMERSON R. COLLINS

(Accepted for publication December 6, 1935)

In previous experiments^{1, 2, 3} it was shown that ammonia in relatively dilute concentrations is toxic to the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar. In additional laboratory tests, conducted in 1934, the mycelium in sectional agar blocks was killed with ammonia water after 24 hours with a concentration of 50 p.p.m. NH_3 ; and the sclerotia from 2-year-old soil cultures in 1 hour with a concentration of 300 p.p.m. Although the above data are of interest from the standpoint of probable utilization of ammonia and ammonium compounds for treatment of areas infested with root rot, it is essential to know the approximate concentration of ammonia in the soil necessary to suppress development of the fungus before applications can be made in the field that may give promise of reducing the disease.

When ammonia is applied to types of soil such as constitute the principal root-rot areas of the Texas black-land belt, one is immediately confronted with the problems of base exchange, adsorption, and nitrification, all of which affect to a greater or less degree the concentration of free ammonia that may be obtained from specific applications. In order to obtain some idea of the concentration necessary in a low-lime Houston clay soil to kill both cotton root-rot mycelia and sclerotia, laboratory experiments involving soil treatments with ammonia water and subsequent inoculations with the fungus were conducted in the spring of 1935. Seven hundred and fifty grams of soil (652 g., dry weight) was used for each concentration of ammonia tested, and the range of concentrations varied from 300 to 1325 p.p.m. in increments of 75 p.p.m. In the 300 to 975 p.p.m. range, the moisture content of cultures was adjusted to 27 per cent dry basis, while in the higher ranges, 1025 to 1325 p.p.m., it was 30 per cent. In obtaining the different concentrations of ammonia water used in the soil treatments, the

¹ Neal, D. C., R. E. Wester, and K. C. Gunn. Treatment of cotton root-rot with ammonia. *Science*, new ser., 75: 139-140. 1932.

² Neal, D. C., R. E. Wester, and K. C. Gunn. Growth of the cotton root-rot fungus in synthetic media and the toxic effect of ammonia on the fungus. *Jour. Agr. Res. [U. S.]* 47: 107-118. 1933.

³ Neal, D. C. Recent investigations of cotton root rot in Texas. (Abst.) *Phytopath.* 24: 838. 1934.

amount of N as NH_3 required for each soil concentration in parts per million was calculated and the equivalent supplied from a 0.5801 N solution of ammonium hydroxide, previously standardized against standard acid (H_2SO_4 with methyl red indicator). The required amount of ammonium hydroxide was diluted with water, in the proper proportions, so that the ammonium hydroxide and water brought the soil to the moisture content given for each series. Each concentration of the ammonia water was evenly distributed throughout each soil culture by mixing with spatulas on a mixing board. After treatment, the soil was then transferred immediately to quart Mason jars equipped with modified lids⁴ to facilitate handling incident to making inoculations. The jars were then plugged with cotton to prevent escape of the ammonia, and 48 hours was allowed to elapse after the soil was treated before making inoculations. Inoculations were made by inserting from 20 to 30 root-rot sclerotia⁵ in the center of each jar, and later firmly pressing the soil around them. Sectional agar blocks of the fungus from fresh cultures also were inserted in the soil of each jar. Jars containing untreated soil but adjusted to the same moisture content (27 and 30 per cent, respectively, dry basis) also were inoculated as controls.

Examinations for evidence of growth of the fungus were made 13 days after inoculation in the 300 to 975 p.p.m. range of concentrations and after 26 days in the 1025-1325 range. Good growth occurred in the cultures with concentrations of 300 to 700 p.p.m.; scant growth at 975 p.p.m.; and no growth at 1025 p.p.m. or in the other higher concentrations employed. After intervals of 26 and 42 days respectively from the dates of treatment, some of the cultures were analyzed for ammonia, nitrate nitrogen, and moisture content, and these results are shown in table 1.

The results show that when ammonia is added to a soil of the above type in the form of ammonia water, its conversion to nitrate is fairly rapid, especially when initial applications are in excess of 825 p.p.m. At these concentrations approximately 30 per cent of the ammonia was nitrified after the 26-day interval. The percentage of conversion also, as was to be expected, was greater in the higher concentrations and with the longer interval preceding analysis. In view of nitrification, the concentration of free ammonia obtained at the time of analysis of the cultures in which the fungus did not grow cannot be considered as the effective concentration for kill. Doubtless it is higher, but further work, using much shorter periods of incubation of the fungus after treatment of the soil, is necessary before this can be determined. With the cultures used in these experiments, however, the effective initial concentration in the soil appears to be in the range of 900 to 1025 p.p.m. This is a marked contrast to the lower concentrations

⁴ Originated by C. J. King, Sacaton, Arizona.

⁵ Sclerotia from soil culture 45-days-old.

TABLE 1.—*Results of analysis of cultures for ammonia, nitrate nitrogen, and moisture content*

Soil treatment ^a	P.P.M.—N as NH ₃	P.P.M.—N as NO ₃	Per cent moisture	Growth of fungus
Group 1 ^b				
Check	17.1	15.1	good
900 p.p.m. N as NH ₃	396.4	248.7	moderate
975 " " " "	407.8	264.3	scant
Check	15.1	10.6	good
Group 2 ^c				
1025 p.p.m. N as NH ₃ ^d	498	499	30.0	none
1100 " " " "	547	542	35.4	none
1175 " " " "	628	522	36.0	none
1250 " " " "	697	517	34.6	none
1325 " " " "	788	483	30.5	none
Check	35	44	33.4	good

^a Results expressed on oven-dry basis.

^b All calculations based on 27 per cent moisture. Analysis made 26 days after treatment of soil.

^c Analysis made 42 days after treatment of soil.

^d Treatments were calculated on the basis of 30 per cent moisture. On the basis of the moisture content at date of analysis they were: 1025, 1146, 1230, 1294 and 1330 p.p.m., respectively.

of ammonia water previously referred to in this paper that were effective in killing root-rot mycelia and sclerotia when direct exposures were made in small tightly stoppered Erlenmeyer flasks.

BUREAU OF PLANT INDUSTRY,
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PHYTOPATHOLOGICAL NOTE

Pathogenic Strains in Ustilago nigra.—In the spring of 1935, ten collections of the black loose smut (*Ustilago nigra* Tapke)¹ of barley were used to inoculate 17 varieties. The smut collections had been increased on Alpha barley in the greenhouse during the winter of 1934-35 and were less than a month old and highly viable at the time of inoculation. Prior to inoculation, seed of the 17 barley varieties was treated by the modified hot-water treatment (5-hour presoak followed by 13-minute treatment at 52° C.) to insure freedom from any previous infestation of smut. The inoculated seed was sown in 5-foot rows in duplicate series in May, 1935, at Ithaca, New York, in cooperation with the New York (Cornell) Agricultural Experiment Station. In July the results shown in table 1 were obtained.

Table 1 shows that the varieties Himalaya (C. I. 1312), Lion (C. I. 923), and Nepal (C. I. 595) differentiated collection 186 from Wisconsin as a distinct pathogenic strain. The other 9 collections were pathogenically similar on this set of varieties.

In the fall of 1935 collections 185 and 186 were used again to inoculate smut-free seed of Himalaya, Lion, and Nepal. The inoculated seed was sown in November, 1935, in a greenhouse at the Arlington Experiment Farm, Rosslyn, Virginia. Collection 185 again failed to produce smut in Himalaya and Nepal but did produce 100 per cent smutted heads in Lion. Collection 186, on the other hand, produced 68.0 per cent smutted heads in Himalaya, 30.0 per cent in Nepal, and only 16.7 per cent in Lion. Each percentage is based on a total of approximately 100 heads.

The greenhouse results, therefore, confirmed the earlier field results in showing that pathogenic strains occur in *Ustilago nigra*.—V. F. TAPKE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

¹ Tapke, V. F. A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Research 25: 491-508. 1935.

TABLE 1.—Percentages of smutted heads in 17 varieties of barley grown at Ithaca, N. Y., in 1935 from seed inoculated with 10 collections of *Ustilago nigra* each from a different State

Number and source of smut collection	Alpha C.I. 959	Club Mari-out C.I. 261	Coast C.I. 2235	Excel-sior viola-ceum C.I. 1248	Gatami C.I. 575	Hann-chen C.I. 531	Hillsa C.I. 1604	Hima-laya C.I. 1312	Hor-deum defi-ciens C.I. 668-1	Hor-deum inter-medium C.I. 4377	Lion C.I. 923	Lyall-pur C.I. 3403	Nepal C.I. 595	Odessa C.I. 934	Pan-nier C.I. 1330	Trebi C.I. 936	White Smyrna C.I. 910
125 New York	33.5	0	10.0	0	0	19.4	0	0	0	2.7	12.1	0	0	37.5	0	41.4	32.0
133 Missouri	20.2	1.1	11.6	0	0	23.5	0	0	0	0	16.4	0	0	57.6	0	43.8	16.5
143 Texas	22.6	0	3.6	0	0	25.7	0	0	0	0	13.8	0	0	57.5	0	29.8	15.6
176 Pennsylvania	32.2	0	7.8	0	0	16.9	0	0	0	0	11.0	0	0	60.1	0	24.5	22.2
179 Ohio	48.3	0	3.2	0	0	25.4	0	0	0	0	15.1	0	0	50.0	0	46.5	23.7
180 Iowa	29.0	0	4.8	0	0	30.1	0	0	0	0	18.4	0	0	60.1	0	43.8	20.6
181 North Dakota	27.0	0	3.9	0	0	33.9	0	0	0	0	35.4	0	0	47.0	0	47.9	25.1
184 Washington	42.0	0	7.6	0	0	31.2	0	0	0	0	30.2	0	0	70.2	0	44.3	34.1
185 Colorado	32.9	0	6.6	0	0	32.5	0	0	0	0	36.9	0	0	60.2	0	43.5	27.5
186 Wisconsin	23.4	0.9	3.4	0	0	15.6	0	16.8	0	0	2.5	0	32.0	47.9	0	32.7	10.0

^a The total number of heads ranged from 146 to 563 in the different varieties.

BOOK REVIEW

Seifriz, William.—*Protoplasm*. 584 pp. 179 fig. McGraw-Hill Book Co. New York and London, 1936. \$6. (36s.)

Right at the outset, the reader of this interesting book on the physiology and morphology of protoplasm should be told that the title is merely a new (and very fitting) one for a book dealing with the principles of general physiology. After all, since the problems of cellular physiology are those of protoplasm, this should not be surprising. And a very instructive book it is, well adapted to the needs of advanced students who are interested in life *per se*, whether of plants or animals.

The first 15 of the book's 27 chapters deal primarily with the physics and chemistry of the protoplasmic stuff, in which field Seifriz is particularly at home. These chapters include a discussion of micrurgy, surface tension, viscosity, etc., with a summarizing chapter on "The structure of protoplasm and organic colloidal matter."

The next 7 chapters consider the cell and protoplasm in relation to the immediate environmental influences, and include discussions of permeability, acidity, electricity, radiant energy, and the rôle of water and salts in life processes.

The last section of the work includes a treatment of the products of protoplasm (carbohydrates, fats, proteins, hormones, etc.) and their inter-reactions with protoplasm. A final, very splendid chapter on "The origin of living matter," which permits the author to express his views on the philosophical problems of biology, closes the volume, not counting the bibliography (arranged according to chapters and authors) and the index (authors and subjects combined). In this last chapter Seifriz reviews the question of vitalism vs. mechanism and enrolls himself among the neo-vitalists (emergent evolutionists). While admitting that he has little hope that protoplasm will ever be manufactured *in vitro*, he urges that "the only safe method of procedure in science is the experimental and mechanistic one" and reminds us that "a severe mechanist may be more humanistic in his attitude towards the world than the most ardent vitalist." He then adds the significant statement that "the difficulty is that we have learned to control nature before we have learned to control ourselves."

In each chapter the author discusses the physical and chemical principles involved, and the book may thus serve as an excellent reference work for a statement of the fundamentals of biophysics and biochemistry. The first 15 chapters, which include the author's own field of research, are especially illuminating in this respect.

Although the book is on the whole well done and easily holds the reader's attention, the style, especially in the first chapters, while the author is

getting the feel of his pen, is in spots jerky, abrupt, disconnected, and even a trifle slovenly. On p. 19, for example, we read of "the Amoeba found in the sublingual spaces of our mouth," on p. 30 of "perpetual young tissue," and on p. 159 of "the initiation of the impetus to surface tension studies which has gone so far in biological reasoning." We also read in reference to tools used in micrurgy (p. 51) that "Certain of them can be bought, some of which are useful and timesaving"; that (p. 143) "The following diffusion constants are of several colloidal and crystalloidal substances"; and (p. 469) "A classified list, with examples, will convey an idea of the kind of substances that proteins are better than will a definition." The readers of the manuscript and proof, as well as the editors, failed in their duty here, or these sentences would not have escaped them. At times the idea expressed becomes almost naïve, as on p. 24 where we learn "It is the function rather than the reality of cell parts which troubles us most."

These defects in style frequently result in a distinct lack of clarity, as on p. 41 where we learn: "These latter swim about awhile as myxamoebae and then possibly fuse or individually grow into myxamoebae or plasmodia, which are the bodies of the plants."

Also on p. 177 we read: "The union between atoms established by the sharing of an electron is primary valence. Adsorption may be of this type and is then said to be *electronic*. It is distinguished from primary valence in that . . ." Is adsorption primary valence or not? Now you see it; now you don't.

Again on p. 178 we find: "More numerous are those looser bonds, which go under the name of *coordination numbers*. These latter are significant in adsorption. Every atom in a crystal of sodium chloride is surrounded by six of the opposite kind." Opposite kind of what?

The story is told that when Jacques Loeb was once criticised for a lack of clarity, he replied: "Arrhenius will understand." It doesn't always take an Arrhenius to understand what Seifriz means, but clarity could have helped even a Jacques Loeb.

The reader who likes a book of science to possess a certain uniformity and regularity (which we like to think Nature approves of) will regret to see names of genera and species only occasionally italicized (p. 450); generally they are not. Also he will wonder why the same name (*e.g.* Myxomycetes) sometimes (p. 40) is capitalized and sometimes not (p. 7) This, of course, is assuming that some virtue may lie in uniformity and consistency.

Also (as in most books) there are a few errors that can be ascribed directly to the printer and proof-reader, but such errors are not of especial importance; they are only annoying.

But not all the "errors," unfortunately, can be placed on the shoulders of proof-readers. Some of these, to be sure, are subject to differences of opinion. The present reviewer seriously questions whether viable *Nelumbo* seed 200 years old have ever been found (p. 6). Also it is possible that *Oxalis stricta* and *Rumex acetosella* will grow in the laboratory at pH 8, but it is doubtful whether they are found growing luxuriantly in nature at this pH. The old "tradition among farmers" (p. 323) that these plants indicate sour soil may still continue to be fostered, even though it is true that such soils also are frequently sterile. Most farmers know, however, that in a majority of cases liming soils where sorrel and "sour-grass" grow will result in marked improvement.

Again, on p. 245, we read that "the red blood cell of amphibians is 'simply a sac' with 'no visible framework' of structure." In view of the presence of the nucleus mentioned, this seems difficult to understand.

Also on p. 531 the idea is expressed that small cells don't have enough room "for any but simple chemical reactions." Even 200 molecules, if of different kinds, would seem to give plenty of opportunity for complicated reactions, and there is little evidence to indicate that the complexity of cell reactions is associated with size of cell. Quite the contrary, a small, independent bacterial cell may be the seat of reactions much more complicated than those in a much larger tissue cell.

In several places lack of proper digestion of the material seems a bit obvious. On p. 220 we learn that high metabolic activity is associated with low protoplasmic viscosity (called unfortunately "consistency" here and there), but in the next paragraph an example is given of mitosis, during the early stages of which there is an increase in viscosity. Surely, during mitosis, if ever, cells are active. Again in the excellent chapter on elasticity we are told (and correctly) that elasticity must not be confused with ductility or extensibility; and yet, on p. 232 the author himself (by aid of the figure) says he is demonstrating elasticity when he is really demonstrating ductility and extensibility. Similarly, on p. 420, we learn (correctly, I believe) that diabetes is associated with acidity, but on the next page we are told that it is alkalinity that accompanies diabetes. Again, on p. 419, we are told that "a fasting animal may lose all its fat and half its protein but not more than one-tenth of its water"; but two pages farther on we read that "during starvation, the largest loss in body substance is water."

In not all his errors, however, is the author so doubtfully inconsistent. Not all cells of mushrooms are binucleate, as is distinctly implied (p. 17); the hollow-ground slide is at the *left* in Fig. 52; in E, Fig. 91 no continuous spiral is evident, as told us on p. 153; an exothermic reaction is not one that "proceeds without elimination of heat" (p. 458); and chlorophyllase is not

the enzyme responsible for forming sugar from CO_2 and H_2O (508), to cite some of the most obvious errors.

Whether there are errors of *omission* will depend much on the individual reader, but, surely, any list of essential elements and their rôles in plant and animal cells should include under Mg its function in the chlorophyll molecule.

I hope the readers of this review will not think this is a poor book. It is, on the contrary, a very good and inspiring book and one that has been needed in English for some time. If Homer nodded, a physiologist should be allowed a wink or two. The errors cited can easily be straightened out in the 2nd edition, which the publishers seem to promise us with their "First Edition" on the title page (or is this merely a guarantee to bibliophiles and bibliopolists?).

Students of phytopathology are realizing more and more the relations between their subject and cellular physiology. While this book does not relate directly to pathology, it bears many allusions thereto, and the pathologist who reads here will find much to interest him. Wise is the man who knows always what he *ought* to be interested in. But, pathologist or not, this work should find a place on the shelves of all who would like to know what makes our wheels go 'round and to know more of the interrelations between chemistry, physics, and biology.—ORAN RABER.

*REPORT OF THE TWENTIETH ANNUAL MEETING OF THE
PACIFIC DIVISION OF THE AMERICAN PHYTO-
PATHOLOGICAL SOCIETY*

The twentieth annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of Washington, Seattle, June 16 to 20, 1936, in affiliation with the Pacific Division of the American Association for the Advancement of Science. Three half-day sessions were held and 24 papers were presented.

The sessions were well attended considering the fact that the place of meeting was in the extreme Northwest. About 30 members and visitors registered, 2 from as far away as Arizona.

At a business meeting the following officers were elected:

President—B. A. RUDOLPH, University of California, Deciduous Fruit Station, San Jose, Calif.

Vice President—C. W. BENNETT, United States Department of Agriculture, Riverside, Calif.

Secretary-Treasurer—L. D. LEACH, University of California, Davis, Calif.

Councilor—J. W. HOTSON, University of Washington, Seattle, Washington.

The papers covered a variety of subjects. The majority of them were of a strictly pathological nature, but several mycological papers were presented. The pathological subjects discussed included viroses and vectors, fungus diseases of fruits, cereals, and trees, and experiments with various fungicides in controlling plant diseases.

H. P. Severin and J. C. Freitag discussed the transmission of western celery mosaic by aphids and the properties of the virus, also the aphid vectors of celery calico. A. B. Hatch discussed mycorrhizal fungi, calling attention to the greatly increased absorbing surface on pine roots induced by these fungi and the fact that trees cannot thrive in infertile soil in their absence. William Newton described the effects on tomato of a particularly virulent strain of the "potato X" virus. L. J. Klotz described an injury to citrus fruits caused by water. H. S. Fawcett and L. J. Klotz reported a new species of *Candelospora* causing decay in citrus fruits. G. A. Huber made a progress report on the control of narcissus and bulbous iris leaf diseases by Bordeaux-penetrol spray, and on the spread and adhesiveness of this spray material. This combination proved the most satisfactory of any materials tried. J. Kienholz and Leroy Childs conducted trials of various copper sprays for peas and apple scab, considering both effectiveness and

tendency to cause injury. A 2-2-2-50 combination of copper phosphate, lime, and bentonite proved best. R. B. Streets discussed attempts to diagnose cotton root rot in soils by screening out the sclerotia, and indicated the apparent usefulness of heavy applications of ammonium sulphate in aiding recovery of fruit and nut trees from root rot. R. Sprague reported on foot rots of cereals in certain coastal valleys of Oregon, and also on some mycological aspects of species of *Mastigosporium* on Gramineae. G. W. Fischer found that certain smut spores taken from herbarium specimens were viable after periods ranging up to twenty-five years. S. M. Zeller outlined studies on bark necrosis and fruit pit of pear, reported simultaneous infection of strawberry by crinkle and yellows, and described a new genus of gasteromycetes. Karl D. Butler described a disease of the inflorescence of the date palm caused by a *Helminthosporium*, and reported studies on ash tree cankers. Several papers, chiefly of mycological interest, were presented as follows: J. W. Hotson, A new species of *Arachniotus* isolated from contaminated milk; R. H. Tschudy, Cultural studies of the genus *Chaetomium*; and studies of several groups of fungi as they occur in western Washington, namely, operculate *Discomycetes* by Leon C. Snyder; the genus *Inocybe* by D. E. Stuntz; Polypores by J. R. Roberts; and *Hydniums* by W. M. Lanphere.

The University of Washington proved a very hospitable host. The campus was beautiful, and everything was done for the comfort and convenience of the visitors. One of the high lights of the meeting was a dinner for all biologists on Thursday evening, which was attended by several hundred, including many pathologists.

The 1937 meeting will be held the third week of June in Denver, Colorado, as a joint meeting with the National Association and the South-western Division.

L. D. LEACH,
Secretary-Treasurer.

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SOIL CONDITIONS IN RELATION TO LITTLE LEAF OR ROSETTE OF FRUIT TREES IN CALIFORNIA

ANTONI KOZLOWSKI¹

(Accepted for publication Nov. 20, 1935)

In an earlier paper (5) the writer referred to two factors that play the most important part in the rosette of fruit trees, namely, certain parasitic fungi and unfavorable soil conditions. The objection of Chandler and Hoagland (1) that "the conclusions reached (by the writer) are founded on a misinterpretation of certain limited observations made in the course of the general investigation and are irrelevant or inconsistent with the well established facts" is not factually supported by their published note (1). It is, therefore, difficult to guess what facts they meant. One cannot find any fact in their publications or in the literature on little leaf or rosette of fruit trees that would contradict the statements (5) made by the writer. In the present paper are included chemical analyses of water extracts from soil of affected orchards that were described in his previous article (5) to furnish on objective corroboration of his conclusions. Those figures need no further interpretation; they speak for themselves.

The fact (5) of the location of certain kinds of fungi in the bark tissues of the rosette twigs throws a new light upon the problem of that disease in this respect, namely, that it reveals some definite microorganisms and not a "virus" or another unknown agent to be responsible for the characteristic symptoms of the disease. These parasites, however, are not very virulent and are, therefore, unable to infect and destroy a tree if the climate be unfavorable for their development and the nutrition of the trees be satisfactory.

The fungi suspected of playing a causal rôle in the development of rosette of fruit trees in California need for their development a humid atmosphere; dry air, for instance, in Arizona (7), inhibits the germination of the spores on the surface of the plant organs, while a cold winter makes hibernation and development of fungi difficult, even if they succeed in penetrating

¹ The chemical analyses of the soil solutions were carried out by Messrs. J. C. Martin and R. O. Overstreet (Tables 1, 2, and 3) and H. L. Hibbard (Table 4), Division of Plant Nutrition, University of California, Berkeley, Calif., to whom the writer expresses his appreciation of their kindness.

the twig tissues. These circumstances explain why the rosette of various fruit trees is limited in the United States to certain warm, highly humid regions or, to be more specific, why it is rather common in the interior valleys of California and does not occur east of the Rocky Mountains. The fact, however, that even in some central regions of California the fruit trees remain healthy all the time, or at least for several years, indicates that the process of infection and the progress of the disease depend not so much on weather factors as they do on soil conditions.

The writer, in his studies of the cause of rosette, had opportunity to investigate affected orchards on three kinds of an alluvial, sandy type of soil, *i.e.*, a deep sand (plum orchard, Kern County), a slightly loamy sand (peach orchard, Merced County), and a loamy sand (apple orchard, Sonoma County), already described (5).

An interesting case of unfavorable soil conditions was found in the slightly loamy sand of the peach orchard, which also contained some lime. That soil was examined in all its horizons as to its microflora, soil colloids, and chemical composition of soil solution. There was found at a depth of about 6 feet a hard layer of calcium carbonate (calcareous) hardpan a few centimeters thick. The horizon of accumulation (illuvium) was located at a depth of about 4 feet; it contained, besides some loam, organic substances, hydrates of sesquioxides, and deflocculated soil colloids in a much larger amount than any other horizon. Although it was still far from being cemented into a hardpan, it was already quite impervious to water and air. Thus, between that calcareous hardpan and illuvium, an anaerobic chamber was formed in which the processes of fermentation were very strongly accentuated, mostly during the rainy season when a fresh supply of organic substances, deriving from the decaying leaves, was brought down. There was found a comparatively large amount of ferrous-ferrie hydrate, which extended to just above the illuvium to a depth of about 90 to 60 cm. from the soil surface. Because of the anaerobic soil conditions, the feeder roots, formed at the beginning of plant growth, became necrotic; the xylem of the roots contained considerable iron. The fibrous roots of diseased trees were scarce and more or less covered with nematode galls. These, as well as the lenticels, weakened by anaerobiosis, exposed the roots to attack by root-invading parasites. The soil temperature, at a depth of about 3 feet, was 22.5° C. on October 24, 1931.

To ascertain the possible presence of toxins, a physiological analysis of the water extracts of that soil was carried out as follows: fresh soil samples, taken separately from each soil horizon, were stirred with an equal quantity of water and allowed to stand over night. The clear supernatant extract was dispensed into a few 1-liter corked jars, each carrying 2 sunflower seedlings about 4 cm. long; a series of 4 to 6 jars was used for the soil extract

of each horizon. Comparatively, the best nutrient value was shown by the soil extracts from the surface horizon and from the lower part of the subsurface horizon (A_2), which covered the horizon of accumulation (Table 1 and Fig. 1). Since no soil toxins could be isolated, a chemical analysis of water extracts of soil samples of various soil horizons was carried out to see whether a deficiency of some nutrient elements was responsible for a poorer growth of the sunflower seedlings in the soil extracts of the subsurface horizon and of the subsoil.

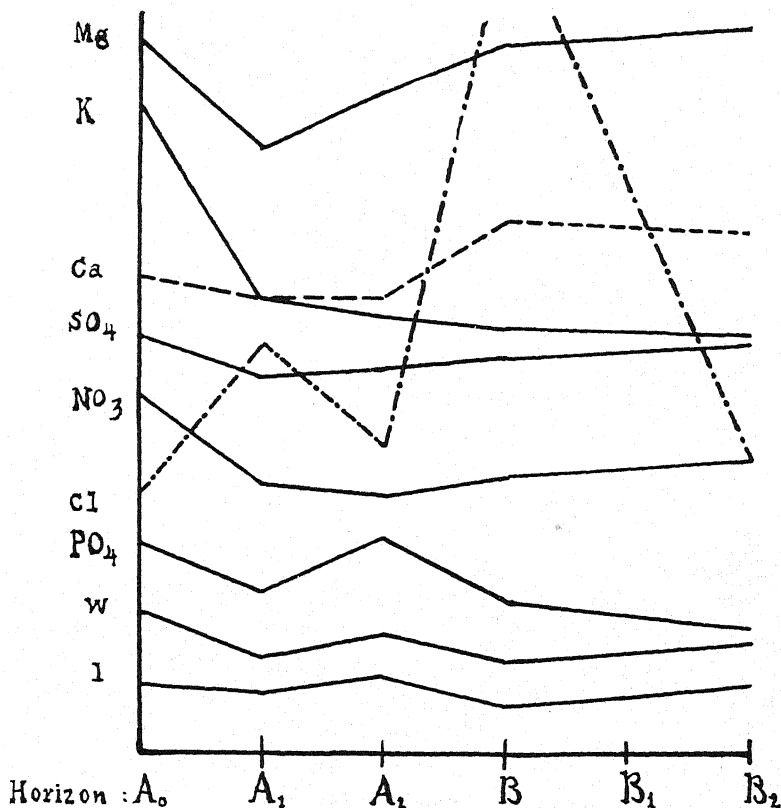


FIG. 1. Curves showing the content of certain chemical elements and of the nutrient value of the soil solution in various soil horizons. W shows average weight of dry substances, l shows average length of the stems of sunflower seedlings, grown for 2 weeks in the soil extracts.

The results of the above analysis show that a chemical composition of the soil solution is quantitatively differentiated according to the physical horizons of the soil profile. It throws also a certain light upon the processes of absorption by soil colloids; a sudden and high rise of the sodium chloride concentration in illuvium indicates that that salt was distributed in the soil

TABLE 1.—*Chemical analysis of the distilled water extracts (1:1) from the soil samples of the peach orchard in Delhi, Merced County. (The soil samples were taken in November, 1933, and the pH was determined by means of a quinhydrone electrode)*

Horizons	Depth (cm.)	pH	Components, p.p.m. in water extracts:							
			NO ₃	PO ₄	SO ₄	Cl	HCO ₃	Ca	Mg	K
A ₀	1~ 30	6.96	18.0	2.15	7.7	2.0	21.0	7.6	3.6	4.8
A ₁	30~ 60	6.96	3.6	1.6	1.4	26.0	32.0	3.9	1.8	1.69
A ₂	60~ 90	7.42	2.0	2.22	2.6	10.0	15.0	3.9	2.8	1.4
B	90~120	7.42	4.7	1.15	3.1	205.0	56.0	16.5	3.3	1.34
B ₂	150~180	8.24	6.0	0.67	3.9	7.0	61.0	14.9	3.7	1.1

not according to the law of diffusion but rather to the law of absorption. This phenomenon is very interesting, and its explanation demands some special studies. The above analysis gave a general picture of the physiological conditions that then prevailed. A strong reduction of nitrates in the subsurface horizon indicated that anaerobic conditions were created even over the horizon of accumulation. By this means one obtained a confirmation of a microbiological soil analysis that revealed a great abundance of the denitrifying bacteria of the *Pseudomonas* group in the rhizosphere of the diseased peach trees at a depth of 2 to 3 feet.

Similar, but much more accentuated, unfavorable soil conditions were developed in a sandy loam of an apple orchard in Sonoma County. Here, too, a very compact subsoil was found at a depth of about 6 feet. The horizon of accumulation had developed at a depth of about 3 feet; it contained a considerable amount of a sticky clay besides hard lumps consisting of sand, loam, and the hydrates of sesquioxides that were cemented together. In this soil, too, there was formed an anaerobic chamber in the subsoil in which processes of fermentation were strongly accentuated, above all during the winter season. Processes of denitrification occurred even above the horizon of accumulation. Sodium chloride was accumulated in the illuvium (Table 2).

The bark of the roots of the diseased trees was in some places corroded, and that on fibrous roots was so completely decayed that only xylem remained.

A physiological analysis of the soil solution showed a very low nutrient value in all soil horizons, possibly because of a comparatively high content of the sulphates of the alkaline earths (Table 2).

A different case was represented by a deep sand in a plum orchard in Bakersfield, Kern County. It contained a small amount of loam, and was very pervious. There was found no hardpan at a depth of 6 feet, and, according to information obtained from Professor C. F. Shaw, there were

TABLE 2.—*Analysis of water extracts (1:1) of soil samples from Sonoma County, California, taken in early February, 1934**

Horizons	Depth (cm.)	pH	Components, p.p.m. (Oven-dry basis):							
			NO ₃	Cl	SO ₄	PO ₄	Ca	Mg	K	Na
A ₀	1- 30	5.55	12.8	7.0	13.8	1.4	8.4	4.0	7.3	2.7
A ₁	30- 60	5.75	2.0	6.5	12.5	0.2	6.0	3.3	5.3	1.9
B	60- 90	5.15	trace	232.0	17.5	0.08	31.5	8.0	16.0	108.0
B ₁	90-120	4.84	7.6	31.0	28.0	0.08	12.1	6.5	3.2	13.2
B ₂	120-150	4.84	7.1	8.0	34.5	0.11	9.4	5.5	2.6	6.4
B ₃	150-180	4.72	7.7	9.0	31.5	0.15	8.0	6.2	1.2	6.7

* pH was determined by means of quinhydrone electrode. Soil moisture was: 7.85 per cent (A₀), 8.6 per cent (B), 12.95 per cent (B₁), 14.2 per cent (B₃).

places in which no compact substratum could be found, even at a depth of 20 feet, while in some other places of that area the ground-water level reached the surface in winter. Thus, the fruit trees were exposed in that orchard to injury by a too strong fluctuation in the soil moisture content, which was very low, even during winter (Table 3). There is then no doubt that the water supply in that orchard was deficient just at the time when it was much needed. It accounts for the death of the ends of the young twigs and the lateral buds. It is possible that, in addition to drought injury, there may have been some injury chargeable to anaerobic soil conditions, because the organic products of leaf decomposition could have very readily percolated down during the winter.

In that soil the illuvium was indistinct and only slightly marked by an accumulation of small amounts of loam. Alkalinity was so pronounced that calcium carbonate incrustated the soil surface. Available nutrient matter was low in the soil solution (Table 3); and the growth of sunflower seedlings was poor in the soil extracts of all soil horizons.

Roots of the plum trees were pretty well developed, although they were often affected by canker.

The above 3 instances of a chemical analysis of the soil solution are given here to illustrate the soil conditions in the rosette-affected orchards described by the writer in his first article. If one abstains here from the discussion whether in those orchards the fruit trees were or were not subject to malnutrition because of mineral deficiency, such as that of zinc (3), in the soil solution or because the latter was not well balanced physiologically, one must, nevertheless, take into consideration some facts established by the soil analyses, namely, processes of denitrification, unfavorable soil reaction, and some unfavorable conditions in the illuvium of the orchards in which anaerobic soil conditions were strongly accentuated. The data in tables 1,

TABLE 3.—*Analysis of water extracts (1:1) of Bakersfield, Kern County soil, California^a*

Depth (cm.)	pH	Components, p.p.m. (Oven-dry basis) :								
		HCO ₃	NO ₃	Cl	SO ₄	PO ₄	Ca	Mg	K	Na
1- 30 ...	8.44	71	7.6	4	7.6	0.69	11.9	3.9	5.35	19
30- 60 ...	8.47	85	1.24	6	9.8	0.07	10.6	2.9	1.85	28
60- 90 ...	8.50	89	0.88	8	15.0	0.05	11.4	3.1	0.98	32
90-120 ...	8.58	87	0.82	6	12.3	0.07	10.6	2.7	0.83	32
120-150 ...	8.60	82	0.92	6	13.6	0.17	8.4	3.3	0.85	30
150-180 ...	8.58	84	0.86	7	14.0	0.23	8.4	3.0	0.90	33

^a Soil samples were taken in the first days of March, 1934. Soil moisture content: 2.75 per cent (1-30 cm.), 5.9 per cent (30-60 cm.), 5.55 per cent (150-180 cm.).

2, and 3 show that in all 3 orchards soil conditions were unfavorable to normal growth of the trees. It is, therefore, not surprising that their resistance to attack by parasites was very low.

The writer attempted to verify Professor Hoagland's soil-toxin hypothesis, taking, however, into consideration also the conditions of soil and of the roots, and aboveground parts of the diseased trees. He found in his various experiments: that a rosette could not be produced either by soil microorganisms or by any product of their metabolism; that a water extract of fresh soil samples from certain soil horizons was less favorable for growth of sunflower seedlings than that from other horizons; that this less favorable condition was not traceable to an assumed soil toxin but to a deficiency of some nutrient elements, and especially of available nitrogen; that the nitrogen deficiency was due to denitrifying bacteria of the *Pseudomonas* group (type *P. fluorescens denitrificans*); and that those bacteria were much more abundant in the soil of affected orchards than in that of orchards in which the trees improved under a soil treatment; that the development of those bacteria was facilitated by anaerobic soil conditions that were distinct in the affected peach and apple orchard (Tables 1 and 2); that the roots of peach and apple trees were very badly injured, the parts aboveground showing some lesions in which were found various microorganisms; little leaves of a rosette were covered by the spores of various saprophytic or facultative parasitic fungi (*Altenaria* sp., *Cladosporium* sp., and some others), and also by numerous kinds of bacteria; and that *Monilia* sp. was found in the bark of rosette-producing twigs.

The results of all those experiments, carried out in the above order, led the writer to conclude that the trees under investigation became so weakened by unfavorable soil conditions that they could no longer resist the attack of parasites invading their roots and aboveground parts.

The hypothesis that "zinc sulphate acts by breaking up or precipitating some injurious and unknown chemical either in the soil or after absorption by the tree, or else by inhibiting the growth of microorganisms capable of producing toxic compounds" (2, p. 559) is not founded on fact, and, in the writer's opinion, is not justified because, notwithstanding various attempts, he was unable to find such toxins either in the soil or in the twigs of the diseased trees, and could not reproduce a rosette condition by any soil micro-organism, directly or indirectly, or by soil extracts and fresh soil samples. That "little leaf is caused by a deficiency of zinc for normal metabolism" (3, p. 261) seemed doubtful to the authors themselves, in view of the fact that ailing trees recover often without any special treatment. The writer may add here that he has found zinc toxic to the roots of barley, even in a concentration of 0.5 mg. of zinc in one liter of nutrient solution, and there could not be found a dose of zinc that might stimulate growth of that plant.

There is no doubt that under certain conditions zinc sulphate, if applied in an adequate quantity, may improve soil conditions because it markedly precipitates the soil colloids and simultaneously changes the hydrogen-ion concentration in the soil. Thus it facilitates a proper soil ventilation, and contributes, by base-exchange and by the action of its anion (SO_4), to an increase of available nutrient elements in soil solution. At the same time, a development of anaerobic conditions and of the processes of denitrification will be inhibited. Such favorable changes in soil conditions were found in an affected peach orchard in Delhi (No. 2), described in this and in another paper, as one can conclude from the results of a chemical analysis of the soil solution summarized in table 4.

TABLE 4.—*Analysis of water extracts (1:1) of Delhi soil, Merced County, California*

Soil sample	pH	Components, p.p.m.:							
		Ca	Mg	K	SO_4	Cl	NO_3	PO_4	NH_4
A	7.4	1.7	1.0	1.0	5.0	8.0	0.6	0.2	0.1
B	5.79	7.5	1.3	4.8	15.0	8.0	2.0	2.0	0.1
C	6.76	6.0	1.7	15.3	30.0	8.0	1.6	1.6	0.0

Soil samples were taken from the same orchard (No. 2) in Delhi (Table 1) on May 10, 1933, at a depth of 2 to 3 feet, and in the vicinity of trees A, B, and C. Soil around tree A was not treated; the tree was very unhealthy, neighboring trees were dead. Soil around tree B was treated with 9 pounds of zinc sulphate in January, 1932. The tree was completely well. The soil of the neighboring trees also received zinc sulphate. Soil around tree C was

not treated. The tree bore an unhealthy aspect. Soil of some neighboring trees had been given an application of zinc sulphate.

Those figures show a distinct improvement of soil under influence of zinc sulphate in regard to the soil reaction and the content of some nutrient elements in the soil solution. (An increase of certain elements in soil solution under the influence of zinc sulphate was observed first by Krauch (6)). The analysis of soil sample C indicates that sulphuric acid, after an absorption of zinc by soil colloids, diffused quite far in the soil, and caused an increase of potassium and phosphorus content in the soil solution. Because of this circumstance, tree C started to recover (was only "partly sick"). Since Hoagland and Chandler (4, p. 271) gave no particulars concerning their method, one cannot explain why they "have not established the existence of phosphate or potassium deficiencies" in that orchard.

Deflocculated soil colloids were abundant in the nontreated soil and were completely flocculated in the treated one.

A spraying of trees with a solution of zinc sulphate (6 pounds) has, of course, the same effect as the soil treatment in some cases.

A favorable effect, were there one, of the action of zinc compounds applied through the holes into the trunk of a diseased tree might be due partly to the "fungicide" properties of zinc, which, however, are very weak, and partly to the circumstance that certain nutrient elements may be released from the xylem and made available to the buds; but, most probably, it may be explained differently.

Thus, after a critical examination of all experiments on little leaf that have been published by Chandler and Hoagland (2, 3, 4), the writer could not find a single fact in support of their hypothesis of a "specific function of zinc" or that would raise any doubt that his conclusions on the influence of faulty soil conditions upon little leaf or rosette of fruit trees in California "are irrelevant or inconsistent with well established facts." After a very careful study of little leaf for nearly $2\frac{1}{2}$ years the writer came to the conclusion that that disease is due not to a supposed single factor, which has not thus far been found, but to the concerted action of various factors, such as climate, soil conditions, and microorganisms.

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BACTERIAL LEAF SPOT OF PRIMULA¹

P. A. ARK AND M. W. GARDNER

(Accepted for publication January 7, 1936)

A bacterial leaf spot of *Primula* occurs rather commonly in ornamental and commercial plantings in the San Francisco Bay region, particularly on *Primula polyantha*, Mill.

SYMPTOMS

The disease is characterized by irregularly circular brown lesions on the older leaves surrounded by conspicuous yellow halos (Fig. 1). The spots average 5 to 8 mm. in diameter and, when abundant, coalesce and kill large areas of the leaf, or even the entire leaf. In the early stages the spots are small and water-soaked, sometimes with a yellowish center, soon becoming brown and definitely delimited. When young lesions were cut across in a drop of water on a glass slide and examined under the microscope, masses of bacteria were observed to ooze out from the cut edges.

PATHOGENICITY OF THE CAUSAL ORGANISM

Cultures from young lesions yielded yellowish to greenish bacterial colonies. Healthy plants were atomized with a suspension of the organism from 48-hour-old cultures, covered with bell jars for 5 days and kept in a cool shady place. Infection developed very slowly and usually 2 weeks to a month were required for the production of conspicuous lesions. Only the older leaves proved susceptible. No infection on young leaves or on flowers was observed. Plants previously sprayed with Bordeaux 2-2-50 remained free from infection.

MORPHOLOGY OF THE CAUSAL ORGANISM

The organism is a short rod with rounded ends, and occurs singly or in pairs. It stains readily with all of the common bacteriological stains and is Gram-negative. The size of the individual cells varies considerably in any given culture, irrespective of media used, or age of the culture. In 24-hour-old cultures the cells measured from 1μ to 3.16μ in length and from 0.51μ to 0.73μ in width. The organism is motile by means of one flagellum at one pole, as demonstrated by Casares-Gil's² method of staining, and thus belongs to the genus *Phytomonas*, according to the classification of the So-

¹ Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

² Plimmer, H. G., and S. G. Paine. A new method for the staining of bacterial flagella. *Jour. Path. and Bact.* 24: 286-288. 1921.

ciety of American Bacteriologists.³ No endospores, capsules, or involution forms have been noted.

CULTURAL CHARACTERS OF THE CAUSAL ORGANISM

Agar Poured Plates. The organism grows well on ordinary beef agar medium and more luxuriantly when 1 or 2 per cent dextrose is added to potato-peptone medium. It produces a greenish and greenish-yellow discoloration in media.⁴ On peptone-beef agar of pH 6.9, colonies were visible after 24 hours' incubation at 22° C., the optimum temperature for this organism. In 36 hours, the surface colonies were round, convex, smooth, glistening, yellowish and 1 mm. in diameter. After 5 days the surface colonies reached 3 mm. in diameter; were smooth and shiny with entire margins, and there was a slight discoloration of the medium. On poured plates of potato-peptone agar containing 2 per cent dextrose, growth was much more abundant than in nutrient beef agar and discoloration of the medium was more pronounced.

Agar Stabs. The organism grew moderately along the line of puncture when deep-stabbed in beef-peptone agar, the best growth being on top in the form of a flat, circular, spreading film. Growth was better on the potato-peptone-dextrose agar.

Agar Slants. On beef-peptone agar, growth was moderate, slightly raised, smooth and yellowish in a reflected light. The medium was greenish-fluorescent in transmitted light. On potato-peptone-dextrose agar slants, growth was more abundant, and after 5 days' incubation at room temperature the color of the medium was Buffy Citrine and the growth was Andover green. After 9 days on potato-dextrose-peptone agar slants, the medium was Hazel and the slant growth dark greenish olive yellow. On potato-peptone-lactose agar slants, the medium after 5 days' incubation at room temperature was yellowish Citrine yellow; and the growth Elm green; after 9 days the medium became Citron green and the growth had a bluish hue.

Gelatin Plates. In 48 hours small, circular, yellowish colonies appeared around which later developed zones of liquefaction.

Gelatin Stabs. The liquefaction was infundibuliform in 2 days and the medium was completely liquefied in 3 weeks.

Potato Cylinders. On steamed potato plugs growth was rapid, light yellow, glistening, and spreading. The plugs had a light brown discoloration and an odor comparable to freshly plowed soil.

Milk. Milk coagulated within 3 days, and no other changes were observed after 2 weeks.

³ Society of American Bacteriologists. Bergey's manual of determinative bacteriology. 3 Ed. 589 pp. The Williams & Wilkins Company, Baltimore. 1930.

⁴ Ridgway, R. Color standards and color nomenclature. 43 pp. 53 col. pl. The Author, Washington, D. C. 1912.

Litmus Milk. Pale blue litmus milk was changed into pink and then white after 3 days when the coagulation took place. No other changes were observed after 2 weeks.

Reduction of Nitrates. No nitrites were demonstrated when the organism was grown in nitrate broth (0.1 per cent potassium nitrate in beef-peptone broth) and tested with Trommsdorf's and sulphanilic acid- α -naphthylamine reagents from 5 to 30 days after inoculation.

Ammonia Production. The organism grown in beef-peptone and beef-peptone-nitrate broths produced ammonium after 5 days, as shown by positive test with Nessler's reagent.

Indol Production. Cultures in Dunham's solution were negative for indol after 7, 14, and 21 days when tested with sulphuric acid and sodium nitrate.

Hydrogen Sulphide Production. No hydrogen sulphide was produced when the organism was stabbed and grown in the lead acetate beef-peptone agar (Difco brand).

Diastatic Action. There was no diastatic action on starch (0.3 per cent starch in beef-peptone agar) when tested after 5 to 14 days with a saturated solution of iodine in 50 per cent alcohol.

Cohn's Solution. No growth occurred in Cohn's solution.

Uchinsky's Solution. Abundant growth had occurred after 24 hours' incubation at room temperature. After 78 hours a greenish discoloration was observed at the top of the test tube, and after 1 week the medium was greenish fluorescent.

Fermi's Solution. Good growth was noted after 24 hours with the formation of a greenish fluorescence of the medium after 48 hours.

*Synthetic Medium According to the Society of American Bacteriologists.*⁵ Good growth developed after 24 hours with greenish discoloration of the medium after 48 hours.

Toleration to sodium chloride. The organism grew very well in beef-peptone broth containing 1 per cent NaCl, fairly well in 2 per cent, weakly in 3 and 4 per cent, and was inhibited by 5 per cent of sodium chloride in the same medium.

Carbon metabolism. To test the ability of the organism to utilize organic carbon compounds, 1 per cent of dextrose, lactose, sucrose, maltose, galactose, arabinose, glycerine, dulcitol, and mannitol respectively, was incorporated into S.A.B. synthetic medium⁵ and incubated at room temperature. Brom cresol purple was used as an indicator. In all compounds tested, an acid but no gas was produced.

Hydrogen-ion Range. By means of adjusting beef-peptone broth with hydrochloric acid and sodium hydroxide, the following hydrogen-ion con-

⁵ Society of American Bacteriologists. Com. on bacteriological technic. Manual of methods for pure culture study of bacteria. 4 Ed. Leaflet II, p. 8. 1930.

centrations were obtained: pH 4; 4.5; 5.0; 5.3; 5.5; 5.8; 6.0; 6.3; 6.5; 6.8; 7.0; 7.5; 8.0; 8.5; 9.0; 9.5; 10.0. The limit of tolerance is between pH 4.5 and 5.0, and best growth occurred at pH 6.8 and pH 7.0.

Relation to Oxygen. The organism grew in the closed arm of fermentation tubes and along the stab when inoculated into beef-peptone-agar deep. Thus the organism is a facultative anaerobe.

Temperature Relations. The organism was inoculated into broth tubes and beef-peptone-agar slants and incubated one week at 1°, 4°, 7°, 10°, 13°, 16°, 19°, 22°, 25°, 28°, 31°, 34°, 37°, and 40° C. Growth occurred between 10° and 34°, and the best growth after 24 hours was at 19° and 22° C.

Since the host plant thrives best in cool climates, it is significant that the organism grows at temperatures as low as 10° C. To determine the thermal death point, a water suspension of the organism was placed in thin-wall agglutination tubes, exposed to experimental temperatures for 10 minutes in a water bath, and cooled rapidly before inoculating fresh sterile broth with a loopful. It was found that 10 minutes' exposure to a temperature of 49.3° C. was lethal for the organism.

Effect of Freezing. To test the effect of freezing, the organism was suspended in sterile distilled water and placed in a cold-storage room at a constant temperature of 0° C. Tubes were frozen solid in ½ hour and thereafter cultured every 24 hours. The organism could not be recovered after 3 days, a fact indicating its inability to withstand exposure at 0° C.

Resistance to Desiccation. Smears made on sterile cover slips, using water suspension of a 24-hour-old agar culture of the organism, were dried for different time intervals; survival of the bacteria then was ascertained by dropping a cover slip into a sterile broth medium, with subsequent incubation at room temperature. Under the conditions obtaining, the organism survived only 60 minutes' drying. The organism survived 6 months in sterilized soil in test tubes.

Vitality in Culture Media. The organism survives 2 months in broth without any transferring and only 2 weeks in potato-peptone-dextrose agar slants.

TAXONOMY OF THE CAUSAL ORGANISM

The causal organism appears to be an undescribed species, since the morphological and physiological characters do not resemble those of any phytobacteria previously described.

*Technical Description of *Phytomonas primulae* n. sp.* A motile rod with rounded ends and one polar flagellum, single or in pairs, measuring in length from 1 μ to 3.16 μ and in width from 0.51 μ to 0.73 μ ; facultative anaerobe;

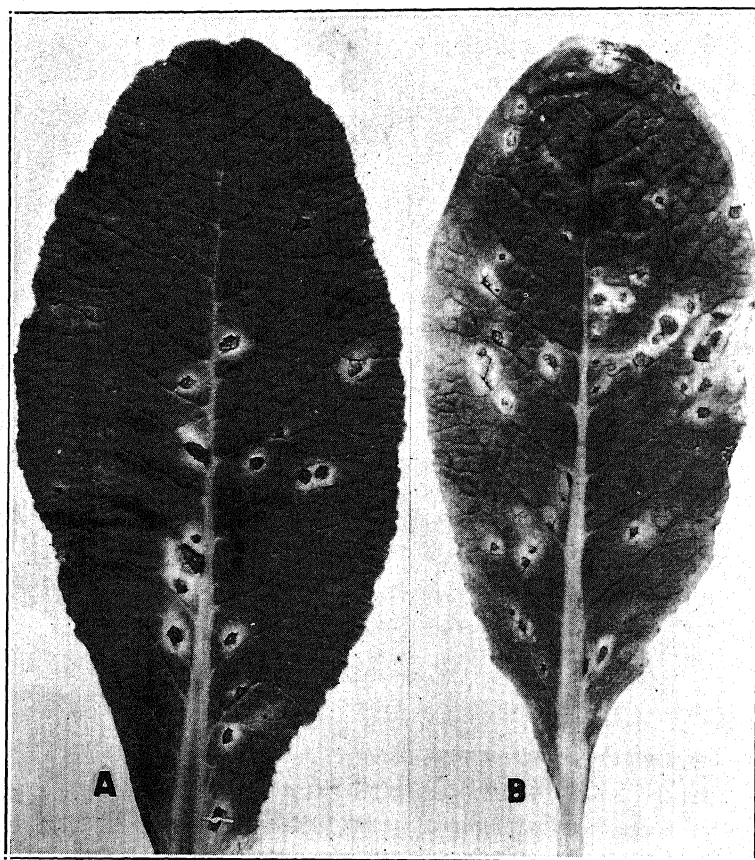


FIG. 1. Bacterial leaf spot of *Primula polyantha*. A. Natural infection. B. Infection produced by spraying the plant with a water suspension of *Phytomonas primulae*, n.sp. Six weeks after spraying.

nonspore forming; noncapsulate. Stains readily with carbol fuchsin, methylene blue, and gentian violet; Gram-negative.

On beef-peptone agar plates colonies are round, convex, smooth, glistening, margin entire; greenish discoloration of medium; produces greenish pigment in S.A.B. medium, Fermi's and Uschinsky's solutions, in beef-peptone and more pronounced in potato-lactose peptone and potato-dextrose-peptone agar; no growth in Cohn's solution. Gelatin liquefied; milk coagulated in 3 days with acid production; nitrates not reduced; indol not produced; ammonia produced; no hydrogen sulphide produced; no diastatic action on starch; acid without gas from dextrose, lactose, sucrose, maltose, galactose, arabinose, glycerine, dulcitol, and manitol. Optimum temperature 10° to 22° C.; maximum 24°; minimum 10° C.; very sensitive to desiccation

SPECIES AND VARIETIES OF PRIMULA FOUND SUSCEPTIBLE

Plants of a number of species and varieties of *Primula* were kindly supplied from the University Botanical Garden by T. H. Goodspeed and P. H. Brydon, for use in inoculation tests.

Infection was obtained on the following: *Primula anisodora* Balf. and Forr., *P. aurantiaca* Sm., *P. auricula* L., *P. carpathica* Fuss., *P. cortusoides* L., *P. denticulata* Sm., *P. denticulata* Sm. var. *cachemiriana* Hook., *P. elatior* Hill, *P. florindae* Ward, *P. forrestii* Balf., *P. malvacea* Franch., *P. obconica* Hance, *P. polyantha* Mill., *P. puchella* Franch., *P. secundiflora* Franch., *P. septemloba* Franch., *P. serratifolia* Franch., *P. sikkimensis* Hook., *P. violacea* Sm. and Ward, *P. vittata* Bur. and Franch., and a hybrid between *P. bulleyana* Forr., and *P. beesiana* Forr.

No infection was obtained upon *Primula japonica* Gray, *P. littoniana* Forr., *P. microdonta* Franch., *P. rosea* Royle var. *grandiflora* Hort., *P. sibirica* Jacq., and *P. viali* Del., ex Franch.

On outdoor plantings of the above mentioned susceptible species and varieties in the Botanical Garden, infection was noted on *P. aurantiaca*, *P. elatior*, *P. polyantha*, and *P. septemloba*. In a lath house, infection was noted on *P. chungensis* Balf. and Ward.

SUMMARY

The bacterial leaf spot of *Primula* is characterized by brown spots with yellow borders on the older leaves.

The causal organism has been named *Phytomonas primulae*, n. sp., of which a technical description is given. Colonies on agar media become green in color. The organism grows well at the low temperatures favorable to *Primulas*.

Among 27 species and varieties of *Primula* inoculated, 21 proved susceptible. Under natural conditions infection has been noted on 5 species, one of which was not included in the inoculation tests. In inoculation tests and in the garden only the older leaves proved susceptible.

INFECTION STUDIES WITH *SCLEROTINIA FRUCTICOLA* ON BRUSHED AND NONBRUSHED PEACHES

M. A. SMITH

(Accepted for publication March 12, 1936)

The extent to which peach pubescence holds spray residue, dirt, and other foreign material is well known. In order to meet the demand for a more attractive pack, the removal of pubescence by means of brushing machines of various types has come into use in many peach packing centers.

Dorsey and Potter,¹ in their study of the structure and pubescence of the peach in relation to brushing, concluded that the peach hair is broken off at the surface of the fruit, either in brushing or handling, because the point of greatest constriction, and, consequently, the weakest point, is just below the surface (Fig. 1, B). They suggest that spores could make a much more direct contact with a brushed than with a nonbrushed surface.

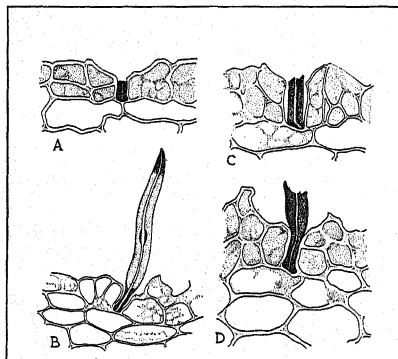


FIG. 1. A, C, and D. Types of breaks in peach hairs as a result of brushing. B. Unbroken peach hair, showing base and the buffer cells of the epidermis.

Because of the type of surface exposed (Fig. 1, A, C, D), it appears that rapid penetration and subsequent infection of the brushed fruit is possible. The work herein reported was carried out with a view to determining the manner in which the brown-rot fungus, *Sclerotinia fructicola* (Wint.) Rehm, infects brushed and nonbrushed peaches, respectively, and to ascertain differences in the ease and degree of infection.

INFECTION STUDIES

Eighty market-ripe Elberta peaches, which had not been sprayed for 30 days previous to picking, were divided into 4 lots of 20 peaches each.

¹ Dorsey, M. J. and J. S. Potter. A study of the structure of the skin and pubescence of the peach in relation to brushing. Illinois Agr. Expt. Sta. Bull. 385. 1932.

In 2 of the lots the excess pubescence was removed by brushes of a texture similar to those used in commercial brushing machines. The remaining 2 lots were not brushed. One lot of the brushed and one lot of the non-brushed fruits were sprayed with a conidial suspension of *Sclerotinia fructicola* and then placed in damp chambers. The 2 remaining lots were not inoculated but were sprayed with distilled water and placed in damp chambers to serve as checks.

Beginning 4 hours after inoculation, and on each succeeding hour, the fruit was examined to note progress of infection. In addition, at the end of 4, 4½, and 5 hours, and for each succeeding hour thereafter, small pieces of tissue were cut from each peach and "fixed" for further study.

As brought out in table 1, in which the results of the inoculation experiments with the brown-rot fungus are summarized, infection was first observed on two of the brushed fruits after a period of 4½ hours. Five

TABLE 1.—Infection of brushed and nonbrushed peaches by *Sclerotinia fructicola*

No. fruit	Treatment	Number peaches showing infection after									
		4 hrs.	4½ hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	9 hrs.	10 hrs.	11 hrs.	12 hrs.
20	Brushed inoculated	0	2	5	11	16	20				
20	Brushed not inoculated	0	0	0	0	0	0	0	0	0	0
20	Not brushed inoculated	0	0	0	0	0	6	13	20		
20	Not brushed not inoculated	0	0	0	0	0	0	0	0	0	0

showed infection at the end of 5 hours, 11 at the end of 6 hours, 16 at the end of 7 hours, and 20 at the end of 8 hours. In the nonbrushed fruits infection was not observed until at the end of 8 hours, when 6 showed infection. The number had increased to 13 at the end of 9 hours, and all were infected by the end of 10 hours. It is apparent from these results that infection occurred more rapidly in the brushed than in the nonbrushed fruit.

HISTOLOGICAL STUDIES

Pieces of tissue that had been removed at hourly intervals from inoculated and noninoculated, brushed and nonbrushed fruit, were removed from the fixative and embedded in paraffin. The sections were cut 6–10 μ thick and stained with a haematoxylin-eosin stain.

Nonbrushed Fruit. In sections from nonbrushed material that had been fixed at intervals of 4½, 5, and 6 hours after inoculation, respectively, conidia could be seen held above the surface of the fruit by the hairs and, where germination occurred, the germ tubes in many cases appeared to extend out in no particular direction. Some conidia were seen resting between hairs with their germ tubes extending to or over other hairs. Occasionally one or more germ tubes were seen appressed to the sides of hairs with their tips pointed in the direction of the hair sockets.

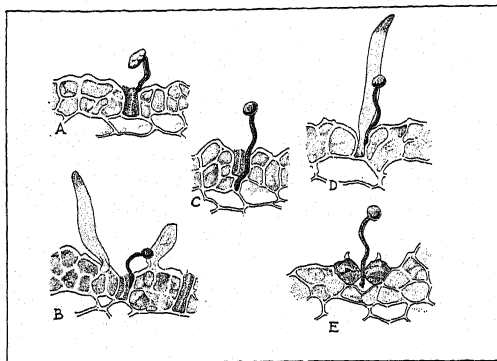


FIG. 2. A. Germinating conidium with tip resting at a point between upper edge of broken hair stub and adjacent buffer cell. B. Germinating conidium resting on short hair with germ tube pushing down between edge of hair stub and adjacent buffer cell wall. C. Penetration of germ tube through point of attachment of hair stub into tissue beneath. D. Germ tube descending hair on which conidium is resting. Note penetration of germ tube into hair socket at hair base. E. Stomatal infection. In A, C, and E spores were pulled away from epidermis during manipulation.

In the material fixed at intervals of 7, 8, and 9 hours, respectively, the course of the germ tubes was more easily traced. By far the larger number of germ tubes could be seen appressed to the sides of the hairs, descending toward the hair sockets, then into the V-shaped depression at the base of the hair socket (Fig. 2, D).

Some germ tubes could be seen extending from one hair to another and then abruptly turning downward along the second hair to its base. Multiple infection through one hair socket was common, and germ tubes were easily traced into the tissue beneath.

Entry of germ tubes through stomata was rare (Fig. 2, E). Several instances were recorded of infection occurring through hair sockets adjacent to uninvaded stomata.

Direct infection through cuticle of epidermal or buffer cells was observed in only one instance.

From these observations it was apparent that by far the larger number of infections in the nonbrushed fruit was by way of the hair sockets, which results are in agreement with those obtained by Curtis².

Brushed Fruit. It would seem that conditions should be favorable for rapid infection on the surfaces of brushed fruit, since conidia that alight on the brushed surface are in very close contact with the short or broken hairs or may actually be lying in broken hair sockets or on buffer cells between hair sockets.

An examination of the material fixed 4 hours after inoculation showed that by this time large numbers of conidia had started to germinate. Measurements of 200 germinated spores at this stage showed that germ-tube length averaged 17 μ .

Sections from peaches that became infected $4\frac{1}{2}$ and 5 hours, respectively, after inoculation showed germinated conidia resting on broken hair stubs with their germ tubes lying parallel to the fruit surface. Others lay on buffer cells with the tips of the germ tubes extending to the ends of broken hair stubs; and in some cases the tips had pushed in between the side of the broken hair stub and the adjacent buffer cell wall (Fig. 2, B). Examination of the material fixed 6 or more hours after inoculation showed that numerous infections had occurred. At this stage the germ tubes were, as a rule, easily traced as they grew downward along the broken hair stubs (Fig. 2, A), between the stub and the adjacent buffer cell to the point of attachment of the stub and then into the tissue beneath (Fig. 2, C).

Stomatal and direct infections, as with the nonbrushed fruit, were rarely seen. An examination of several hundred slides showed an average of 40 infections by way of broken hair sockets to 1 directly through the epidermis or through a stoma.

While hair-socket infection was also the predominant type in the nonbrushed fruit, it was apparent that infection of the brushed fruit occurred more promptly and that the rapidity with which this infection occurred was correlated with the relative accessibility of the brushed fruit surface to germinating conidia of *Sclerotinia fruticola*.

SUMMARY

Methods of infection of brushed and nonbrushed peaches by *Sclerotinia fruticola* were studied.

Infection of the nonbrushed surface of the peach variety Elberta occurs mainly by way of the hair sockets. The minimum period for infection was 8 hours.

² Curtis, K. M. The morphological aspect of resistance to brown rot in stone fruit. Ann. Bot. [London] 42: 39-68. 1928.

The most common method of infection of brushed fruit was by way of the broken hair sockets. The minimum time for infection was $4\frac{1}{2}$ hours. Stomatal and direct infections were not common either in the brushed or the nonbrushed fruit.

The rapidity with which infection occurs on the brushed fruit is apparently correlated with the relative susceptibility of the brushed fruit surface to germinating conidia of *S. fructicola*.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
SPRINGFIELD, MISSOURI.

EFFECT OF LEAF-HOPPER YELLOWING UPON THE CAROTENE CONTENT OF ALFALFA¹

HOWARD W. JOHNSON²

(Accepted for publication June 2, 1936)

INTRODUCTION

Second-cutting alfalfa throughout the Eastern and Mid-western United States is yellowed and stunted to a greater or less extent each summer by attacks of the potato leaf hopper, *Empoasca fabae* (Harris). In a previous paper³ the writer reported that yellow alfalfa is lower in nitrogen and higher in carbohydrates than green alfalfa kept free from leaf hoppers. Since the protein content of alfalfa is one of the chief measures of its quality, this represents a loss that growers suffer in addition to the obvious reduction in yield. Recent research has shown that the vitamin A activity of animal roughages is another important measure of their quality. Since the carotene content of plant tissues is now recognized as a measure of their potential vitamin A activity, an experiment was planned to secure data on the effect of leaf-hopper yellowing upon carotene content of alfalfa.

EXPERIMENTAL METHODS AND RESULTS

Samples for the writer's previous analytical work were produced in infested and noninfested cages of cheesecloth or tobacco cloth. To avoid the altered light and moisture conditions to which caged plants are subjected, it seemed wiser in this experiment to adopt some method of protecting certain field plots of alfalfa, while leaving others unprotected. The most convenient method of protection appeared to be dusting with a mixture of 300-mesh sulphur and pyrethrum, as recommended by DeLong⁴ for the control of this insect on beans and potatoes.

The alfalfa plots used were sown in September, 1933, and were in their second cutting season at the time of this experiment. The plots were cut

¹ This paper reports the results of one phase of a cooperative study of the injury by *Empoasca fabae* (Harris) to forage legumes being made by the Division of Cereal and Forage Insect Investigations, Bureau of Entomology and Plant Quarantine and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

² Indebtedness is acknowledged to C. A. Cary and H. G. Wiseman, Bureau of Dairy Industry, U. S. Department of Agriculture for making the carotene determinations.

³ Johnson, H. W. Nature of injury to forage legumes by the potato leafhopper. Jour. Agr. Res. [U. S.] 49: 379-406. 1934.

⁴ DeLong, D. M. The relative value of Bordeaux mixture, sulphur and pyrethrum products in reducing populations of the potato leaf hopper (*Empoasca fabae* Harris). Jour. Econ. Ent. 27: 525-533. 1934.

on May 28, 1935. Starting on June 18, when recovery growth was well under way and slight yellowing was evident, one plot of Grimm alfalfa was dusted once each week for 4 consecutive weeks, while a duplicate plot was left unprotected. By July 9, when the last dust application was made, the unprotected alfalfa was severely yellowed and stunted, while the dusted alfalfa was green and vigorous. This difference is shown in figure 1, which illustrates the dusted and nondusted alfalfa on July 15, 1935. On July 17, 300 gram samples from the two plots illustrated in figure 1 were weighed in the field and plunged immediately into a



FIG. 1. Plots of second-cutting Grimm alfalfa growing at Arlington Experiment Farm, Rosslyn, Virginia, on July 15, 1935. Both plots were cut on May 28 and, starting on June 18, the plot on the right was dusted once each week for 4 consecutive weeks with a mixture of 300-mesh sulphur and pyrethrum, while the plot on the left was left unprotected. The dusted alfalfa is green and vigorous, while the nondusted is severely yellowed and stunted by a heavy infestation of potato leaf hoppers.

freezing mixture of absolute alcohol and solid carbon dioxide. The samples thus prepared were taken to the Nutrition Laboratory, Bureau of Dairy Industry, for carotene determinations.

The plots were cut for the second time on July 19, 1935. At this time 3- to 4-pound samples were placed in cloth bags and taken to a hay drier. When thoroughly air-dried, these samples were ground in a Wiley mill and stored in Kraft bags until December 12, 1935. On this date samples of the meal made from the dusted and nondusted Grimm alfalfa were tested for carotene. The determinations were made spectrophotometrically. The results of the two sets are presented in table 1.

It is seen from the first set of determinations in table 1 that the fresh green alfalfa contains 262 mg. of carotene per kilo of dry material, while the leaf-hopper-yellowed alfalfa contains only 105 mg. The second set of determinations show that after approximately 5 months' storage in Kraft bags in a hot attic, the meal made from green alfalfa still contains 50 mg. of carotene per kilo of dry material, while the carotene content of the meal made from yellowed alfalfa is only 28 mg. per kilo. While loss of

TABLE 1.—*Effect of leaf-hopper yellowing upon the carotene content of second cutting Grimm alfalfa at Arlington Experiment Farm, Rosslyn, Virginia in 1935*

Plant material	Condition	Moisture	Carotene ^c
		<i>per cent</i>	<i>mg.</i>
Green alfalfa ^a	Fresh 7/17/35	72.83	262
	Dried, ground and stored 12/12/35	9.10	50
Yellowed alfalfa ^b	Fresh 7/17/35	72.33	105
	Dried, ground and stored 12/12/35	9.60	28

^a Dusted four times with a mixture of 300-mesh sulphur and pyrethrum between first and second cuttings.

^b Not dusted.

^c Per kilo of dry matter.

carotene apparently has proceeded more rapidly in the green alfalfa under these storage conditions, yet this material still retains an amount slightly exceeding the average for U. S. No. 1 alfalfa hay, as reported by Meigs.⁵ It would appear from these determinations that severely yellowed alfalfa contains only about one-half the carotene that is present in green alfalfa. This represents a further serious annual loss that must be attributed to the activities of the potato leaf hopper.

SUMMARY

Samples of green and leaf-hopper-yellowed Grimm alfalfa were taken from a plot dusted 4 times with a mixture of 300-mesh sulphur and pyrethrum between the first and second cuttings and from a similar plot left unprotected during this period.

The fresh green alfalfa contained 262 mg. of carotene per kilo of dry matter while the leaf-hopper-yellowed alfalfa contained only 105 mg.

After 5 months' storage in Kraft bags in a hot attic, meal made from the green alfalfa contained 50 mg. of carotene per kilo of dry matter, while the meal made from the yellowed alfalfa contained only 28 mg.

It would appear from these determinations that leaf-hopper-yellowed alfalfa contains about one-half the carotene in green alfalfa and is, therefore, only about one-half as rich in potential vitamin A activity.

DIVISION OF FORAGE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

⁵ Meigs, E. B. Vitamin A value of plant feeds fully accounted for by their carotene content. U. S. Dept. Agr. Yearbook 1935: 324-326.

A SIMPLE DEVICE FOR RECORDING THE TIME AND DURATION OF RAINFALL

RAY R. HIRT¹

(Accepted for publication February 29, 1936)

During epidemiology studies it is often desirable to obtain, automatically, records of the time and duration of rainfall directly at the experimental area. When the location of such areas prohibits the use of electric power, either due to the absence of power lines or inconvenience in bringing in batteries to run electrically driven instruments, mechanically actuated devices must be resorted to. An ombroscope may serve such a purpose, but those described in the literature^{2,3} are difficult to construct because of their complicated designs. While conducting studies concerned with the relation of meteorological conditions to the natural infection of white pine by *Cronartium ribicola* Fischer, a simple, inexpensive instrument was devised to record automatically the time and duration of rainfall. Because this instrument was so easy to construct and worked so efficiently, it was felt that it might prove useful in other research projects and therefore justified a brief description.

The device consisted of a revolving disk actuated by the mechanical parts of a standard alarm clock and the whole mounted in a suitable wooden case (Fig. 1). The wooden case was 9 inches square by 2½ inches deep and was provided with a removable cover fashioned from heavy roofing paper. Legs, 2 inches long, were attached to the bottom of the box in order to raise it slightly above the ground or other substratum. Several quarter-inch holes were bored in the bottom of the box to permit rain water to drain away, and the entire box was painted with a waterproof varnish. The clock works were removed from their original metal case and mounted in the wooden box in such a manner that the winding key and set key projected out through the bottom. A strip of thin rust-resistant metal 2 inches wide was fastened about the clock works, so that it simulated the metal sides of the clock before the works were removed. A thin brass disk, 8 inches in diameter, was then substituted for the hour hand (Fig. 1).

¹ This work was done by the writer while employed as agent in the United States Department of Agriculture, Division of Forest Pathology, in cooperation with The New York State College of Forestry, Syracuse, N. Y.

² Fergusson, S. P. The ombroscope, an instrument for determining the time and duration of rain. Quart. Jour. Royal Meteorological Soc. 31: 313-316. 1905.

³ Covert, R. N. Meteorological instruments and apparatus employed by the United States Weather Bureau. Jour. Optical Soc. Amer. and Rev. of Scientific Instru. 10: 345-346. 1925.

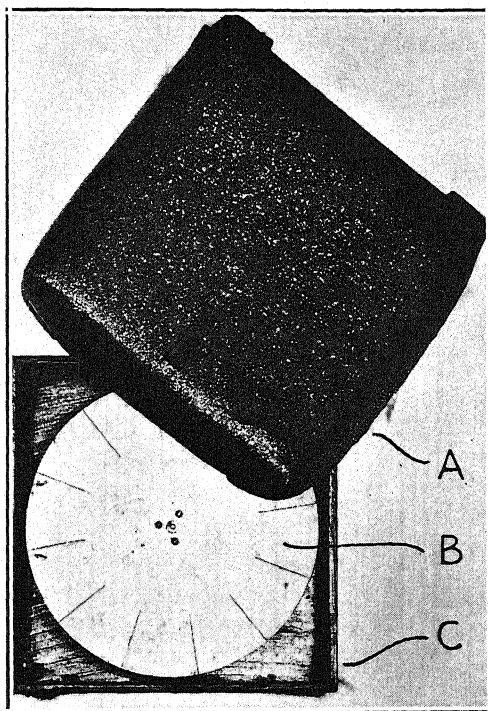


FIG. 1. The rain-recording instrument with its cover removed. A. The cover showing the slit through which rain drops may fall. B. The revolving brass disk upon which the record sheets are attached. C. The wooden box within which the mechanical parts are mounted.

Record sheets (Fig. 2), which lacked the outer concentric circles, were mimeographed on hard bond paper. Three concentric circles were then drawn in with indelible lead near the outer edge of each record sheet. Each sheet was divided into 12 equal sectors by means of heavy radial lines. Thus, when placed on the revolving brass disk, it took each sector 1 hour to pass beneath any given stationary point. The sectors were bisected with lighter lines in order to represent half-hour periods. The lines were numbered counter clockwise. The record sheets, which were of the same diameter as the brass disk, were held in position on the disk by means of 2 paper clips.

A slit 2 inches long was cut in the cover of the box directly over the revolving disk such that the outer edge of the slit corresponded with the edge of the disk. Each end of the slit was made equal in length to $1/48$ of the circumference of that part of the disk directly beneath it. Hence it took an interval of 15 minutes for 1 radial line of the record sheet to

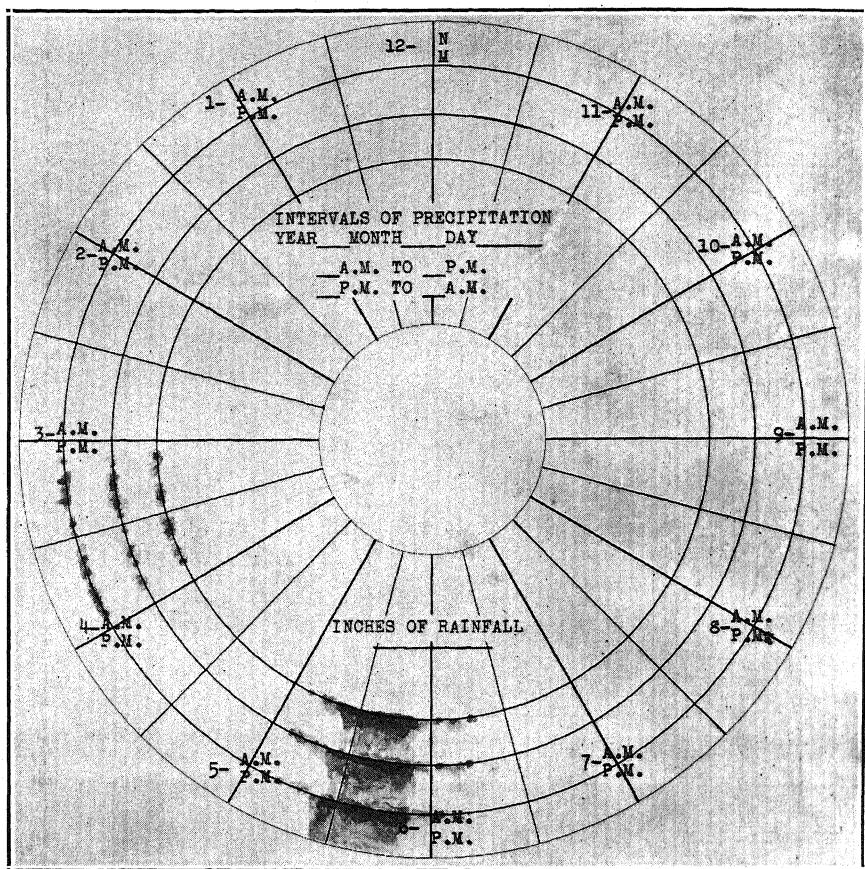


FIG. 2. A record sheet for the rain-recording instrument. Between 3 and 4 o'clock is an illustration of the record left by a light rainfall. The record of a heavier rainfall between 5 and 6 o'clock appears as a streaming of color from the arcs of the 3 concentric circles made with indelible lead.

pass beneath this opening in the cover. When it rained, the water drops fell through the hole in the cover and stained the indelible lines on the portion of the record sheet that at that time was exposed beneath it. The record of the duration of rainfall appeared as colored dots for light rains and as washed streaks of color for heavier rains (Fig. 2). The maximum time-error which might occur for any interval of rainfall was 15 minutes at the beginning and end of the period of precipitation. However in the particular investigation for which this instrument was devised, it was sufficient to know that rain did or did not occur within hourly intervals. By changing the record sheets once every 12 hours and indicating upon each sheet the time when it was placed in the instrument, a complete record of the time and

duration of any rainfall was secured. The amount of rainfall for a 12-hour period was obtained from the reading of a standard rain gauge and noted on the proper record sheet.

The recording device was placed in such a position that it tipped slightly forward; thus no water collected upon the paper sheets but ran off at once, leaving a clearer and sharper record than would otherwise be obtained. It is essential to have the cover of the box raised sufficiently above the disk so that water drops clinging to the edge of the slit will not extend to the revolving record sheet and moisten the indelible lines after the rain has ceased. It is obvious that the accuracy of the records will be increased directly in proportion as the opening in the cover through which the rain drops fall is made narrower.

This instrument has been used for 2 years and is still in good working condition.

NEW YORK STATE COLLEGE OF FORESTRY,
SYRACUSE, N. Y.

THE ISOLATION OF A TOXIC SUBSTANCE FROM THE CULTURE FILTRATE OF TRICHODERMA^{1, 2}

R. WEINDLING AND O. H. EMERSON

(Accepted for publication Jan. 17, 1936)

The parasitic action of the fungus *Trichoderma lignorum* (Tode) Harz on *Rhizoctonia solani* Kühn and other soil fungi has been described in previous papers,³ and some of the properties of a lethal principle that is instrumental in this action have been studied.⁴ This is a preliminary note on the isolation of a crystalline constituent of the lethal principle produced by a culture of *Trichoderma*.

In general, the methods of obtaining and testing the material toxic to *Rhizoctonia* were the same as those used before.⁴ A nonpigmented culture was used, however, since the lethal effects of its culture filtrate lasted longer than those of the pigmented culture previously studied. The "shaking-culture" method described by Kluyver and Perquin⁵ proved very useful in preparing large amounts of potent filtrate.

The toxic effect of this filtrate on *Rhizoctonia* can be removed by extraction with chloroform in a separatory funnel. The solvent is conveniently used in three portions, each equal to 0.1 of the volume of the filtrate. After distilling the chloroform from the extract, the residue is taken up in a small amount of hot benzene, or 95 per cent alcohol, from which, on cooling, silky white needles crystallize. This material is most satisfactorily recrystallized from benzene or alcohol.

The factors that determine the amount of crystalline material produced are not yet completely recognized. This problem is complicated by the decomposition of the material during and after growth. The highest yields (up to 70 mg. per liter of filtrate) have thus far been obtained when the fungus was grown for 2 days under the following conditions: strong aeration, high acidity of the medium (pH 3.0–4.0), ammonium tartrate as the nitrogen source.

¹ Paper No. 341, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

² This research was supported by a grant in aid from the National Research Council to H. S. Fawcett, under whose supervision it was carried out.

³ Weindling, R. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopath.* 22: 837–845. 1932.

⁴ Weindling, R. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopath.* 24: 1153–1179. 1934.

⁵ Kluyver, A. J., and L. H. C. Perquin. Zur Methodik der Schimmelstoffwechseluntersuchung. *Biochem. Ztschr.* 266: 68–81. 1933.

The substance in the crystalline form obtained in these studies is lethal to *Rhizoctonia* hyphae up to a dilution of 1 in 300,000 parts, which is about $\frac{2}{3}$ the toxicity of HgCl_2 under the conditions of these tests. A yellowish brown gum obtained upon evaporating the mother liquors to dryness has varying lethal effects, the maximum being the same as that of the crystals. In addition, it inhibits the growth of *Rhizoctonia* hyphae up to a dilution of one in three millions. The gum showed no tendency to crystallize, and has not been further investigated. The crystals, as well as the gum, are toxic to *Trichoderma*, but the minimum lethal dose is about 40 times that for *Rhizoctonia*. The dilution series for testing the substances has been made from alcoholic solutions, since the toxic substances are very sparingly soluble in water. Alcohol alone is not toxic at the dilutions used.

Composition and Properties of the Crystalline Substance. The substance is a compound of carbon, hydrogen, nitrogen, sulphur, and oxygen, possibly $\text{C}_{14}\text{H}_{16}\text{N}_2\text{S}_2\text{O}_4$. The determination of its molecular weight has offered some difficulties owing to the insolubility of the substance and its instability at higher temperatures. At 165°C . it begins to decompose, forming a yellow oil, and at about 185°C . it melts. The decomposition point of the substance has been determined according to a method devised by Bruce.⁶ This method consists of determining the lowest temperature at which a given substance decomposes within 1 second after insertion of the melting-point tube into the bath. The results from this method agree ((within 1 or 2 degrees) with those obtained on the Dennis bar.⁷ The decomposition point of the crystals after one crystallization from ethyl alcohol was 217° , and 221°C . after one crystallization from benzene. The same materials after 3 successive recrystallizations from alcohol or from benzene had decomposition points of 219° and 222°C ., respectively. The substance is moderately soluble in acetone and chloroform, less soluble in hot benzene and in hot ethyl alcohol, and still less soluble in hot methyl alcohol. It is sparingly soluble in cold alcohol and ether. It is soluble in water, although to a very limited extent. It is strongly levorotatory, $[\alpha]_D^{19} = -239^\circ$ in chloroform.

The substance is easily altered. In alkaline solution it instantly decolorizes potassium permanganate with the formation of a green color. In spite of its nitrogen content, it has no basic properties. To 5 mg. dissolved in a few drops of warm acetone, was added $\frac{1}{2}$ cc. of 0.5 N hydrochloric acid; on cooling, the substance separated unchanged. After 15.2 mg. were boiled for one hour in 5 cc. of 3 N hydrochloric acid and extracted with chloroform, 13.5 mg. of a yellowish gum were recovered, but none of the original material could be crystallized from benzene or alcoholic solutions of this gum. 15.7

⁶ Bruce, W. F., *Organic synthesis*. (In press.)

⁷ Dennis, L. M., and R. S. Shelton. An apparatus for the determination of melting points. *Jour. Amer. Chem. Soc.* 52: 3128-3132. 1930.

mg., dissolved in 15 cc. of ether, was shaken with 3 cc. of 1 per cent aqueous potassium hydroxide, then washed free of alkali. The ether-soluble residue weighed 15.0 mg., showing that almost nothing had been taken up by the alkali. However, afterwards it was possible to isolate only 5 mg. of crystals, indicating that this mild treatment had caused some alteration. Boiling with 5 per cent aqueous potassium hydroxide rapidly splits out sulphur, as shown by the formation of a black precipitate of lead sulphide upon the addition of a few drops of lead acetate solution.

Composition Found by Analysis. C 47.75 per cent, 48.07 per cent;⁸ H 4.36 per cent, 4.43 per cent;⁸ N 8.25 per cent, 8.27 per cent;⁸ S 19.4 per cent.⁹

Composition Calculated for $C_{14}H_{16}N_2S_2O_4$. C 49.38 per cent; H 4.73 per cent; N 8.23 per cent; S 18.82 per cent.

Optical Rotation. $[\alpha]_D^{19} = -\frac{2.23 \times 1000 \times 3}{28} = -239^\circ$.

Molecular Weight Determination. Diphenyl amine was the only substance available in which the toxic crystals were reasonably soluble, and the melting point of which was low enough to insure no decomposition. Even diphenyl amine, at its melting point, did not dissolve more than about 6 per cent of its weight of the crystals.

In 24.8 diphenyl amine, 0.90 mg. substance lowered the melting point $0.9^\circ C$.¹⁰

Molecular weight. $\frac{100 \times 0.9 \times 86}{24.8 \times 90} = 347$.

Calculated for $C_{14}H_{16}N_2S_2O_4 = 340$.

CITRUS EXPERIMENT STATION AND GRADUATE

SCHOOL OF TROPICAL AGRICULTURE,

RIVERSIDE, CALIFORNIA.

INSTITUTE OF EXPERIMENTAL BIOLOGY

UNIVERSITY OF CALIFORNIA,

BERKELEY, CAL.

⁸ By A. Schoeller, Berlin.

⁹ By Kurt Eder, University of Illinois.

¹⁰ Value of 86 for molecular freezing point lowering of diphenylamine from: Chemical Rubber Company, Cleveland. Handbook of Chemistry and Physics. 20th ed. Chemical Rubber Publishing Co., Cleveland, O. 1935.

APPLE POWDERY MILDEW

C. E. B E R W I T H

(Accepted for publication Feb. 10, 1936)

Among the distinctive characteristics of the powdery mildew of the apple, caused by *Podosphaera leucotricha* (E. & E.) Salm., is the far greater severity of the disease in districts on or near the Pacific coast of North America, than at corresponding latitudes in the central and eastern parts of that continent. It has been of interest, therefore, to study some of the conditions affecting the germination of conidia and the inception of infection and to examine under local conditions in California the mode of perennation heretofore studied in detail only by Woodward¹ in England.

GERMINATION OF CONIDIA

Relation of Temperature. Woodward states that the conidia germinate best between 10° and 15° C. (up to 3 per cent in water), while 20° to 22° C. is most favorable for growth of the germ tubes.

The percentage germination in the writer's experiments has been low at best, as was the case with earlier workers. Dry storage of conidia for periods of 1, 2, and 14 days at temperatures from 1 to 10° C., did not seem to affect viability as indicated by subsequent germination (1 to 6 per cent) at 20° C. When freshly collected conidia were shaken immediately onto dry glass slides in moist chambers at different temperatures, a fairly definite optimum was indicated at 19° to 22° C. (Table 1). Each figure in the table represents a count of 100 spores for each of 10 trials or 1000 spores for each temperature. At other temperatures below and above those shown in the table there was no germination.

TABLE 1.—Percentage germination of conidia at different temperatures

Temperatures °C.		7	10	13	16	19	22	25	28	31
Time interval	6 hrs.	0	1	1	2	3	3	2	0	0
	48 hrs.	0	1	1	2	4	5	2	0	0

Relation of Humidity. Conidia placed on dry glass slides in dry containers at temperatures from 1° to 34° C. failed to germinate in any case. The conidia at 1° C. were viable after 2 weeks, while those at 31° and 34° were shrivelled after 6 hours. The spores held at 13° and above for 24 hours did not germinate when exposed to a favorable humidity and temperature.

¹ Woodward, R. C. Studies on *Podosphaera leucotricha* (Ell. & Ev.) Salm. I. The mode of perennation. Brit. Mycol. Soc. Trans. 12: 173-204. 1927.

In another experiment, conidia on dry slides were placed in chambers over such concentrations of sulphuric acid as to maintain relative humidities of approximately 1.4, 30, 70, 90, and 100 per cent. Only the last of these permitted germination. Barium chloride and other chemicals in solution were used in a similar way with similar results, no germination occurring at humidities of 90 per cent or below. It is apparent then that a high atmospheric humidity is essential for the germination of conidia. However, when conidia were mounted in hanging drops of water, the germination, if any, was very low, never over 1 per cent. In raised drops of water no germination was obtained. Likewise, when conidia were mounted in hanging drops of water containing extracts of apple leaves or fruits, orange juice, sucrose (1 and 5 per cent), or Czapek's solution (1 per cent) no germination resulted, while controls in distilled water gave 1 per cent germination.

INFECTION OF APPLE LEAVES

Young seedling apple leaves were detached, inoculated with conidia, and placed with the petioles in vials of water in chambers at different temperatures and humidities. The humidities were maintained as indicated above by the use of different concentrations of sulphuric acid.

No infection was obtained with any humidity at 1° to 10° C. or at 28° C., or at favorable temperatures when the humidity was 90 per cent or lower. At 13°, 16°, 19°, 22°, and 25° C., and at approximately 100 per cent humidity, infection was evident in 45 to 48 hours and conidiophores were produced in 5 days.

Relation of Age of Leaf and Wounding to Infection. To study the relation of age of leaf to susceptibility, leaves were marked soon after emergence from the terminal bud and inoculated at different intervals thereafter. With this method, and using rather slow-growing seedling plants, fair to good infection was obtained on leaves inoculated 1 day after marking, but none on leaves inoculated after 5 days. By abrading the leaf surface with powdered carborundum² prior to inoculation, infection was obtained on the abraded portion of leaves (8 of 10 plants) inoculated 9 days after marking, but not elsewhere on the same leaves. It is apparent that the resistance of leaves increases rather rapidly with increasing age and that this resistance can be partially broken down by slight abrasion of the leaf surface.

PERENNATION

Many investigators of this disease have held the view that the perithecia play little or no part in the annual cycle of the fungus and that it survives the winter in or on the buds. It is only recently, however (Woodward),

² Rawlins, T. E., and C. M. Tompkins. The use of carborundum as an abrasive in plant virus inoculations. (Abstract) *Phytopath.* 24: 1147. 1934.

that a comprehensive study of this problem has been made. The evidence against the participation in the cycle by the perithecia, though of a negative sort, is nevertheless impressive and seems now to be conclusive. Several attempts in the course of this work to germinate the ascospores, both in the presence and absence of apple leaves, were unsuccessful, as were the similar efforts of earlier workers. On the other hand, the mycelium, haustoria, and sometimes conidia are readily demonstrated to be rather deep-seated in the resting terminal and lateral buds of shoots that have become completely invested by the fungus. Both free-hand and microtome sections were made from buds (mostly Yellow Newtown) collected in several different years in July, September, November, December, and February. The fungus is often seen midway or below in the interstices of the buds of the sort that regularly open in spring, with all parts completely covered with mildew. The appearance of the mycelium in the buds during the winter months suggests that the fungus probably survives in the mycelial form.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

THE RELATION OF THE AGE OF NEEDLES OF *PINUS STROBUS* TO INFECTION BY *CRONARTIUM RIBICOLA*

WALTER H. SNELL

(Accepted for publication April 15, 1936)

The question of the susceptibility of white pine needles of different ages to *Cronartium ribicola* Fisch. is of interest not only academically and scientifically, but also in epiphytological and phenological studies. In the West it appears to have been established beyond question that in *Pinus monticola* not only are the needles more susceptible to infection in their second season (when they are a little over a year old), but also, that a plurality, if not a majority, of the cankers occur on wood that was a year old at the time of infection (2). In the East, on the other hand, where *Pinus strobus* is the tree concerned, there is some question in this matter, with the result that it has been impossible with certainty to give definite dates in the epiphytological history of the disease or to correlate waves of infection with meteorological data in phenological studies.

Many, if not most, of the workers in the East believe that the same condition holds for *Pinus strobus* as for *P. monticola*—that one-year-old needles are more susceptible to the fungus than are new needles in their first year of activity. This may be true, but in the writer's opinion, the question has not yet reached the incontrovertible stage. On the other hand, the writer is not ready to say that the results presented herewith give the final answer. He does feel, however, that they cannot lightly be dismissed and that they must be satisfactorily explained before the opposing view becomes established. It is hoped that, at the least, these results will provoke caution against becoming dogmatic about the matter, until enough evidence one way or the other is presented satisfactorily to answer the question.

RESULTS OF INOCULATIONS OF POTTED SEEDLINGS, NORTH CONWAY, NEW HAMPSHIRE

In the early days of white pine blister rust investigations by the Office of Forest Pathology, the writer assisted with the inoculation of hundreds of potted white pine seedlings in New Hampshire. These seedlings, ranging in age from one to 10 years and mostly under 5 years, were collected in the woods and transferred with very little disturbance of the root system to paper cups. Various methods of inoculating with sporidia were used, and when a satisfactory technique had been developed, the success of inoculation was consistently between 50 and 100 per cent.

The conclusions regarding these experiments were reported as follows. In the inoculations of 1918 and 1921 seedlings, if anything, there were more

individual infections on current season's needles than on fully grown leaves (5, p. 149), and again, "77 per cent of the first-year seedlings became infected, whereas only 58 per cent of the older ones (most of which were over 3 years old) became infected" (6, p. 502). Colored plate I of this latter article shows about equal susceptibility of the two ages of needles. Of the 1922 inoculations, seedlings of all years except the first were successfully inoculated, thus contradicting the 1921 results (6, p. 505).

Clinton and McCormick (1) found that a larger percentage of 1-year-old seedlings than older ones became infected as the result of their inoculations.

RESULTS OF THE INOCULATION OF OLDER NATURAL PINES,
NORTH CONWAY, NEW HAMPSHIRE

In 1922, at North Conway, N. H., pines from 5 to over 30 years of age, growing undisturbed in their natural habitat, were inoculated. These results have been reported (4). As to the susceptibility of the needles, it was stated—"None of the inoculations of 1920 needles, 18 per cent of those of 1921 needles and 19 per cent of those on the 1922 needles developed leaf spots. None of the inoculations on 1920 needles, 9 per cent of those on 1921 needles, and 8 per cent of those on 1922 needles resulted in twig cankers" (6, p. 507). The pertinent conclusions from the examination of 127 other cankers were as follows (*loc. cit.*):—"Ninety-five per cent of these cankers were on 1922 wood and 5 per cent on 1921 wood. These results confirm the above statement that both 1- and 2-year-old needles are susceptible. They do not, however, give any information on the relative susceptibility of 1921 and 1922 needles, for no record was kept of the relative percentages of the 1921 and 1922 originally inoculated. The most that can be said is that no indication was encountered that 2-year-old needles of *Pinus strobus* are more liable to infection than 1-year-old needles."

RESULTS OF INOCULATIONS OF PLANTED PINES AT NEWCOMB, NEW YORK

Beginning in 1929, for 4 seasons the writer made inoculations of pines set out at Newcomb, N. Y., for the purpose of testing the resistance of the offspring of an apparently immune parent tree. The trees were 6 years old when the first inoculations were made and 9 years when the last ones were done. In these tests infected black currant leaves were wrapped around the needle-bearing portion of the pine twigs and fastened in place. The entire needle-bearing portion of a branch was so treated. Thus, in every unit inoculated, there were needles of current, second, and third seasons of activity, and in some cases, some of 4 seasons.

In all, 389 test and control trees were inoculated and about 56 per cent became infected. The data on the relative susceptibilities of needles of different ages are given below in terms of infection of the internodes and number of cankers an internode.

TABLE 1.—*Data on inoculations of planted pines at Newcomb, New York*

Age of needles inoculated	Internodes			Cankers	
	Total no. inoculated	No. infected	Per cent infected	Total no. cankers	Ratio of cankers to no. internodes inoculated
Current season	3034	416	13.7	497	1 to 6
Second season	1392	163	11.7	184	1 to 8
Third season	448	36	8.0	40	1 to 12
Fourth season	58	3	5.0	3	1 to 20

In these experiments, the year-old needles were somewhat less susceptible than those of the season current with the inoculation, and the ratio of cankers to internodes inoculated was lower, the older the needles at the time of inoculation.

OBSERVATIONS OF NATURAL INFECTIONS ON THE HEWITT AREAS IN NEW YORK

The so-called "Hewitt Areas" in New York are those parcels of land acquired by the State under the Hewitt Act of 1931 for afforestation purposes. In the course of studies concerning the blister-rust control program on these areas over the southern tier of counties from the Catskills to Lake Erie in 1934, observations were made on the cankers found, which were necessarily few in number because of the eradication of *Ribes*.

The trees on most of the areas studied were planted in 1930 or 1931. These studies were made in the summer of 1934, before 1934 infections could be seen. Hence, the cankers studied could have resulted from infection in one of only 4 years at the most, and in one of 2 years, in some cases. One or 2 areas planted in 1932 were searched—areas in which the only opportunity for visible cankers to have had their inception could have been in 1932—but on these areas, unfortunately, there were only very few cankers to be found, perhaps a half dozen to several thousand trees examined. The criteria used for determining the year of origin of the cankers were as follows.

(A) If on any year's wood there was only discoloration of the bark, it was considered to be due to the 1934 growth of the fungus, and, therefore, the canker originated in the summer of 1933. Such discolorations on 1933 wood were from $\frac{1}{8}$ to $\frac{3}{4}$ inch long. Here it may be realized that it is possible that discolorations on 1932 wood were of 1932 instead of 1933 origin, as they are here considered, because the infection spots on the needles may have been so far from the twig that it took a year for the fungus to reach the stem.

(B) If on any year's wood there were only two zones (a) discoloration (1934 growth of the fungus)

canker was considered to be of 1932 origin. These cankers measured from $\frac{1}{8}$ to $2\frac{1}{8}$ inches, with one 3 inches and another $3\frac{1}{8}$ inches long.

(C) If on any year's wood, there were three zones (a) discoloration (the 1934 growth of the fungus) (b) a pycnial zone (the 1933 growth) and (c) old pycnial scars and/or aecial scars of 1934 (1932 growth of the mycelium), then the canker was considered to have originated in 1931. These cankers were found to measure from 3-9 inches in length.

On the basis of the foregoing criteria, the cankers found were distributed as shown in the following table.

TABLE 2.—*Distribution by years of cankers on Hewitt areas in New York*

Year of wood on which canker was found	Stage of development of canker		
	Discoloration only ($\frac{1}{8}$ " to $\frac{1}{4}$ ")	Discoloration and pycnial zones (mostly $\frac{1}{8}$ " to $2\frac{1}{8}$ ")	Discoloration, pycnial and aecial zones (3" to 9")
1933	10	—	—
1932	7	71 (current 1932 infections)	—
1931	2	51 (1932 infections of 2nd season needles)	330 (current 1931 infections)
1930	—	14 (1932 infections of 3rd season needles)	145 (1931 infections of 2nd season needles)
1929	—	—	41 (1931 infections of 3rd season needles)

The foregoing data may be presented in another form, as follows.

Year in which infection took place (origin of canker)	Season's needles infected		
	Current	Second	Third
1933	10	7	2
1932	71	51	14
1931	330	145	41
Totals	411	213	57
Ratio	7.21	to 3.72	to 1

EXPOSURE OF POTTED PINES TO NATURAL INFECTION AT
TUPPER LAKE, NEW YORK

In 1931, 17 pines, potted that spring, were exposed during August underneath some cultivated black currant bushes at Tupper Lake, New York.

They were then transplanted for observation, to gardens in Warrensburg, New York, and Providence, Rhode Island, where there was little likelihood of occurrence of further infection.

TABLE 3.—*Results of exposure of potted pines to natural infection at Tupper Lake, New York*

Location of pines after transplanting, and number	Current season's needles		Second season's needles	
	Internodes infected	No. cankers	Internodes infected	No. cankers
Warrensburg (12)	33	54	38	65
Providence (5)	10	10	0	0
Totals	43	64	38	65

The year-old needles of the 12 pines left at Warrensburg were somewhat more susceptible than the first-season needles. Why there should be the difference between the 2 lots of pines is difficult to understand, for they were all exposed at the same place for the same period, and the 5 removed to Providence were selected at random. The pines taken to Providence apparently were not placed in such a favorable situation, for they did not grow well and died within 2 or 3 years. Why cankers should have developed on the internodes of the current season of inoculation and none on the year-old internodes of the pines at Providence is likewise a mystery, unless the pines were in such a poor location that the needles became weakly and, therefore, inhibited the mycelium of the fungus from reaching the stems.

DISCUSSION

From the foregoing results of observations and experiments of different sorts over more than 15 years in different localities, it can be seen that in most cases the young needles of the first season turned out to be at least somewhat more susceptible to infection by the blister-rust fungus than the year-old needles of the second season of growth. The writer is not, however, insistent upon maintaining the implied thesis. The numbers of plants were, in most cases, small. The observations were, in some cases, limited in scope. The technique may, in some cases, have been faulty or unreliable. There is always a suspicion of artificial inoculations and a question as to the value of the results of these in explaining natural phenomena. The least that can be said, however, is that one should go slowly in advocating the opposite thesis with regard to *Pinus strobus* until valid criticism can show that these results are unreliable or until results to the contrary are uniformly obtained from inoculations and observations under natural conditions.

In connection with inoculation experiments with potted pines, one possible criticism should be avoided in the planning of the work. By this is meant the possibility of objection to the use of pines potted in the same season in which they are to be inoculated or exposed. It is readily observed that transplanted pines do not make such vigorous growth in the season in which they are transplanted, if they make any considerable growth at all. New growth in such pines may be 3 to 5 inches long, whereas the growth on the same trees of the season before, or the season following, would measure 6 to 9 inches in length. The needles on the new wood in the season of transplanting are invariably shorter than before or after. It seems to have been demonstrated that the blister-rust fungus is more likely to produce the disease in vigorous than in weakly growth (3, p. 483). Therefore, until evidence to the contrary is produced, it may always reasonably be objected that the current growth of pines transplanted in the same season in which the experiments are carried on, may possibly have its susceptibility to *Cronartium ribicola* reduced by its lack of normal vigor.

It just so happens that the only set of experiments set forth in this paper, in which the second-season needles were more susceptible than the first-season needles (in the potted pines exposed at Tupper Lake and transplanted at Warrensburgh, New York), was concerned with pines potted in the spring of the same season in which they were exposed to infection.

SUMMARY

It is still an open question whether the first-season needles of *Pinus strobus* are more or less susceptible to infection by *Cronartium ribicola* than the second-season needles (one-year-old).

The results (1) of the artificial inoculation of potted white pines, of older pines in their natural environment and of young planted pines, (2) of the exposure of potted pines to natural infection, and (3) of observations of cankers on young planted pines, are to the effect that the current season's needles are to a greater or less degree more susceptible to *C. ribicola* than the second season's needles.

It is suggested that, in experiments planned for the purpose of settling this question the use of pines potted in the same season in which they are to be inoculated or exposed to natural infection, may justly be open to criticism.

BROWN UNIVERSITY,
PROVIDENCE, RHODE ISLAND.

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PHYTOPATHOLOGICAL NOTES

Sclerotium rolfsii as a Disease of Nursery Apple Trees.—In August, 1935, some yearling apple trees with diseased roots were received from a Maryland nursery for diagnosis of the trouble. By means of the usual culture methods a pure culture of *Sclerotium rolfsii* was readily obtained. Diseased apple trees from other nurseries also yielded the same fungus. From apparently diseased root tissue taken later in the summer, however, cultures were obtainable from only a very small percentage of the material, if at all. In one case, material from which the *Sclerotium* fungus had been previously isolated and that still showed macroscopic characters of the disease, gave negative results for cultures, and, when placed in moist chambers, developed sclerotia in only 1 of 30 roots. Repeated efforts to isolate the fungus from trees whose roots had been dead and left undisturbed in the soil for a month or more, gave negative results. This seems to indicate that, frequently, the causal fungus dies soon after killing the host. The early dying of the fungus in the tissues it has killed suggests the possibility that the disease may be of more frequent occurrence than has hitherto been suspected, and that failure of the writer and other workers to isolate the fungus more frequently may be due to its early disappearance.

The following experiment was conducted to test the correctness of the field observation on the pathogenicity of this fungus. On February 15, 1936, roots from apple nursery stock were inoculated with a pure culture of *Sclerotium rolfsii* by placing about them sclerotia growing on un-hulled oats that had previously been inoculated. The apple roots were then packed in moist sphagnum and held at room temperature. At the expiration of a month, 18 of the 20 roots were infected with *S. rolfsii* and extensive killing was in evidence about the infections. From these diseased roots, a fungus similar to that used as inoculum was reisolated. This test confirms the field observation that *S. rolfsii* readily and rapidly kills the roots of young apple trees when conditions are favorable for its growth.

The disease on apple nursery stock usually may be recognized in the early stages by a web of white mycelium on the tree at the soil level and on the soil around the tree. Within a few days the white mycelium disappears and masses of brown sclerotia (Fig. 1) about the size and color of mustard seeds may be seen on the collar of the tree and on the soil immediately adjacent to the tree. By the time sclerotia are formed the bark of the tree is killed about the collar, effecting complete girdling, which soon results in death of the top. The fungus is confined largely to root tissue, though it may advance up the stem and kill it slightly above the surface of the soil. The bark, especially of roots, is the main tissue invaded and is very quickly killed.

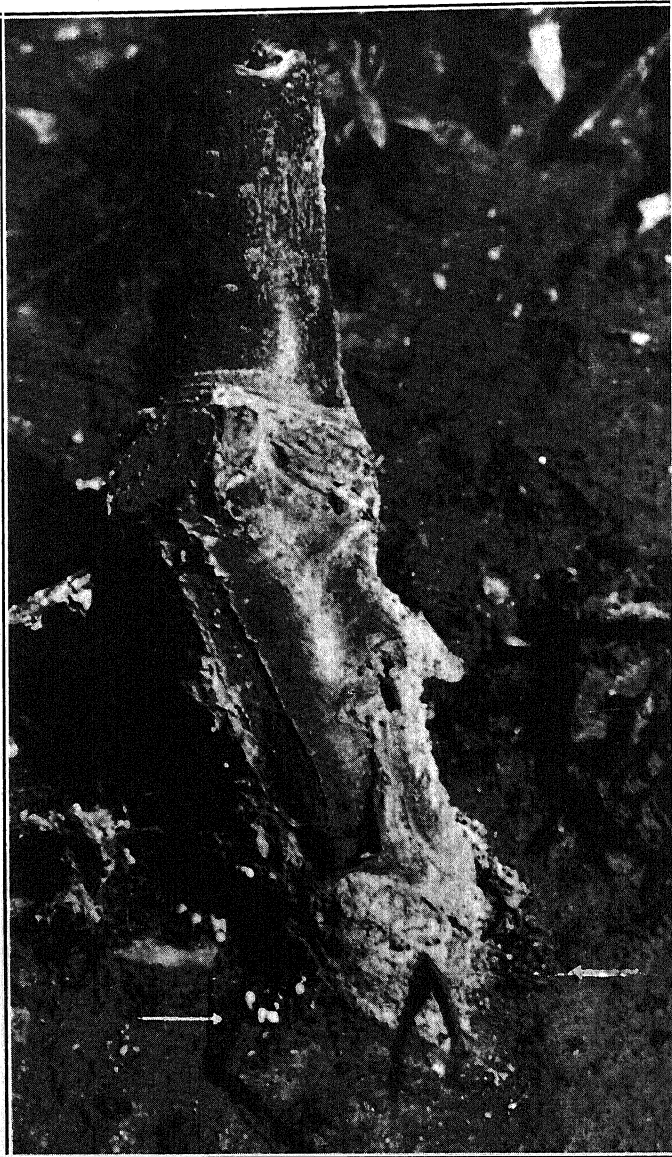


FIG. 1. Budded apple tree girdled in the nursery row by *Sclerotium rolfsii* during the first summer. Arrows point to sclerotia (mature at right, immature at left).

Observations thus far indicate that the disease is more frequent on relatively moist than on dry soil. Trees killed by this fungus do not occur in large groups but are scattered here and there over the affected area. This is probably due to limited distribution of sclerotia at the proper place on the

plant for infection. Considering the fact that the disease has come to the writer's attention in several widely separated apple nurseries during the summer of 1935, it should be recorded as a nursery disease.

Under certain conditions, there may be an appreciable loss of young apple trees. In one nursery, losses have occurred for the past 8 years from a disease apparently the same as that now known to be associated with the *Sclerotium* fungus disease—the losses amounting to as much as 5 per cent of the trees.

Turner¹ has reported the occurrence of *Sclerotium rolfsii* on young apple trees in the orchard.—J. S. COOLEY, Bureau of Plant Industry, U. S. Department of Agriculture.

*Invasion of Cotton Seed by Bacterium malvacearum.*¹—The percentage of cotton-seed contamination due to invasion by *Bacterium malvacearum* E. F. S. is usually low in dry weather, but, under favorable conditions, the seedling infection resulting from internal contamination may reach 20 per cent or more. The bacteria gain entrance to the mature seed through the basal end at the chalaza.

The ovule is surrounded by two seed coats observed by Barrit,² Brown,³ Reeves and Valle.⁴

If a delinted cotton seed that has been soaked in water is placed under a low power microscope, a circular opening through a portion of the outer seed coat at the chalazal end can be seen (Fig. 1, A). The part of the seed coat exposed by this opening is a cap-like structure composed of pigment-bearing cells and large intercellular spaces. This structure is a portion of the layer designated by Brown (2) as pigment-bearing tissue.

While the seed is immature, the smallest intercellular spaces of the cap are .9 μ wide, but by the time the seed has ripened and dried, the spaces have increased to 7.6 to 19 μ wide.

The following tests were designed to determine the porosity of the intercellular spaces of the basal cap:

Test 1. Untreated cotton seeds were soaked in eosin water. Examination of the seed showed that the path of entrance of the eosin-strained water was through the basal cap.

¹ Turner, T. W. Pathogenicity of *Sclerotium rolfsii* for young apple trees. (Abstract) *Phytopath.* 26: 11. 1936.

² Approved for publication by the Oklahoma Agricultural and Mechanical College Experiment Station.

³ Barritt, N. W. Structure of the seed coat in *Gossypium*. *Ann. Bot.* 43: 483-489. 1929.

⁴ Brown, H. B. *Cotton*. 517 pp. McGraw-Hill Book Co., N. Y. 1927.

⁵ Reeves, R. G. and C. C. Valle. Anatomy and microchemistry of the cotton seed. *Bot. Gaz.* 93: 259-277. 1932.

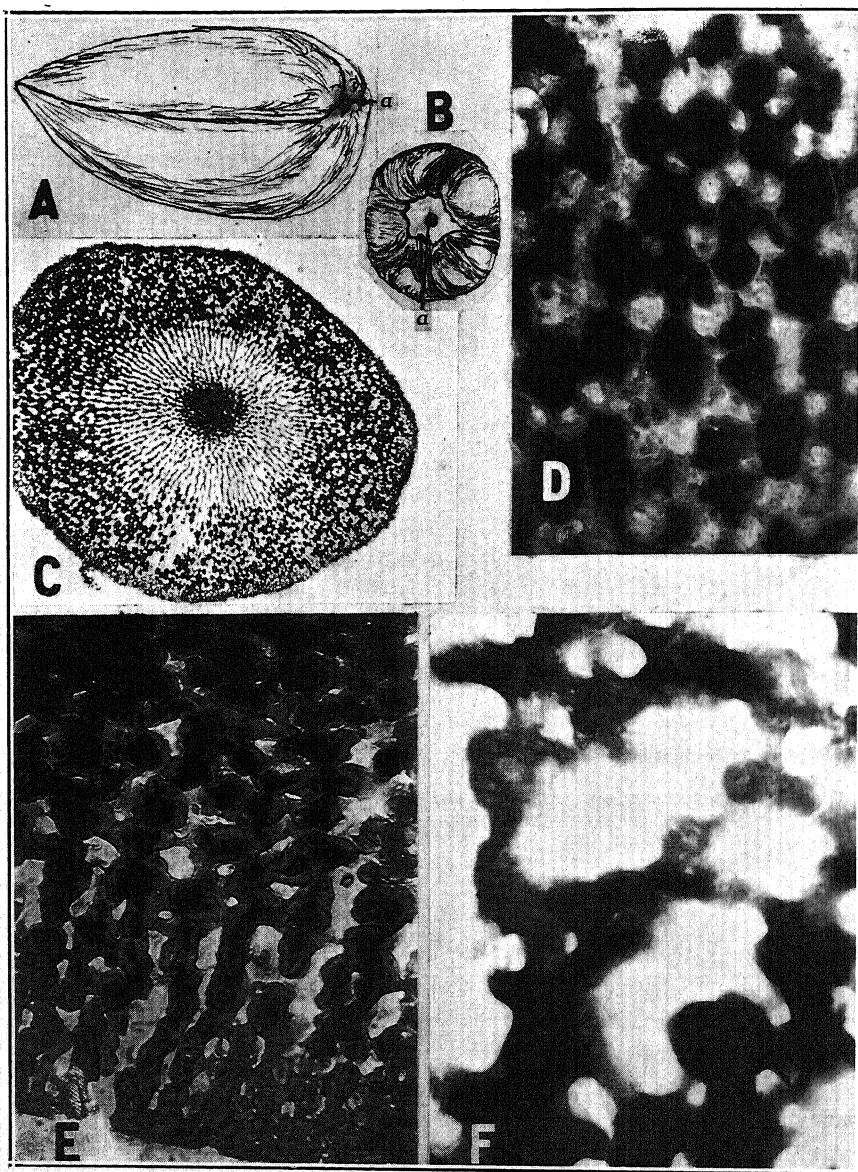


FIG. 1. A. Side view of delinted and water-soaked cotton seed, showing rupture in seed coat at chalazal end and exposed basal cap (a). $\times 4\frac{1}{2}$. B. End view of delinted cotton seed, showing the basal cap (a) at the chalazal end under extreme conditions of pulling apart of the outer seed coat. $\times 4\frac{1}{2}$. C. Cross section of cap. $\times 100$. D. Enlarged portion of C. $\times 430$. E. Longitudinal section through cap structure. $\times 430$. F. Enlarged portion of E. $\times 900$.

Test 2. Delinted cotton seed, with the chalazal end uppermost, were treated with water-soluble starch for 16 hours. Sections of the tissue from the cap were treated with potassium iodide. The starch grains were seen lodged underneath the cap and in the intercellular spaces.

Test 3. This test was conducted in the same manner as test 2. *Bacillus subtilis* in a water blank was used instead of starch. Sections from these seeds showed the bacteria in the intercellular spaces of the cap and along the perisperm layers of the inner seed coat.

Test 4. *Bacterium malvacearum* was used to inoculate the seed. Sections from these seeds showed bacteria underneath the basal cap and in the intercellular spaces of the cap.

Having determined that bacteria could enter the seed through the basal cap in the presence of water, it is necessary to know how and where the conditions exist for seed contamination to take place. This is explained by the fact that rain washes innumerable bacteria to the ground from the foliage of infected leaves. Millions of the organisms are suspended in the run-off water between the rows of cotton.

Evidence shows that the organism remains active in the run-off water for a period of 44 to 56 hours.⁵ Tests show that the seed of open bolls soaked in bacteria-laden water carry both internal and external contamination.

When once the bacteria enter the mature seed, they apparently become inactive and remain so until the germination processes set in. The basal cap adheres to the cotyledons and is carried up as the plant emerges from the soil. The soil water may carry the bacteria to the seed leaves and hypocotyl, or rain and dew may wash the organism onto the leaves and stems, thus inducing primary seedling infection.—GERTRUDE TENNYSON, Oklahoma Agricultural and Mechanical College, Stillwater, Okla.

Fusarium lateritium v. *fructigenum* in Relation to Wilt of China Aster.—A recent article by Riker and Jones¹ described studies at the University of Wisconsin on the pathogenicity to China aster, *Callistephus chinensis* Nees, of various *Fusarium* strains from this country and abroad.

One of the strains from Germany was called *Fusarium lateritium* Nees v. *fructigenum* (Fr.) Wr. This strain was furnished and identified by H. W. Wollenweber who numbered it 4221. The first transfer was received in Wisconsin in the fall of 1930. Subsequent trials, always started by direct transfer from the culture obtained from Germany, proved not only that it was pathogenic to China aster but that the aster strains, susceptible and

⁵ Rolfs, F. M. Bacterial leaf spot of cotton. Oklahoma Agr. Expt. Sta. Rept. 1930-32: 280-283.

¹ Riker, R. S., and L. R. Jones. *Fusarium* strains in relation to wilt of China aster. Phytopath. 25: 733-747. 1935.

resistant with respect to the type strain of *F. conglutinans* Wr. v. *callistephi* Beach, were respectively susceptible and resistant with respect to 4221. A fresh transfer of 4221 was requested and received from Germany in the spring of 1932. It behaved similarly to the first transfer.

Recent examinations by W. C. Snyder and H. W. Wollenweber of 4221, as carried in culture both in this country and in Germany, have suggested that the original 4221 was a mixture of *Fusarium lateritium* v. *fructigenum* and *F. conglutinans* v. *callistephi*.

This new evidence makes it probable that the results obtained in the use of 4221 were due to *Fusarium conglutinans* v. *callistephi*. Consequently, the paper by Riker and Jones¹ should be so interpreted. The *Fusarium* strains shown to induce a wilt of aster essentially similar to that caused by the type strain of *F. conglutinans* v. *callistephi* are, therefore, all classified, according to standard methods, in the section *Elegans*.—REGINA S. RIKER, University of Wisconsin, Madison, Wis.

Viability and Longevity of Chlamydospores of Ustilago crameri.—Chlamydospores of *Ustilago crameri* Kcke. will germinate even before they are morphologically mature, and some have retained their viability for at least 62 years. These facts were obtained in investigating the development and cytology of the organism. To determine the ability of very young spores to germinate, they were taken from sori of various ages, beginning with the emergence of the millet head until the host plant matured. Germination tests were made both in water and 2 per cent sugar solution. A considerable number of spores germinated before they were morphologically mature, when there was still a considerable amount of mycelium in the gall and spore mother cells and spores were present in about equal quantities. Obviously, no rest period is necessary, and the spores appear to be physiologically mature even before they are morphologically so.

It has been known that chlamydospores of *Ustilago crameri* would retain their viability for several years. Hoffman and von Liebenberg, according to de Bary,¹ stated that they retained their viability for 5½ years, and Vasey² found them viable after 3 years.

The writer tested the longevity of spores stored at 7° to 9° C., 16° to 20° C., and 20° to 25° C. A fairly high percentage retained their viability for three years. Tests also were made of spores taken from Rabenhorst, *Fungi Europaei*, Cent. 19, 1874. In several tests about 1 per cent of these spores germinated, indicating that they had retained their viability for at least 62 years. It is probable that the spores actually were 64 years old, as they

¹ Bary, A. de. Comparative morphology and biology of the fungi, mycetoza, and bacteria. 625 pp. Oxford, 1887.

² Vasey, H. E. Millet smuts and their control. Colo. Agr. Exp. Sta. Bul. 242. 1918.

apparently had been collected by R. Körnicke at Poppelsdorf, Germany, in 1872.—C. S. WANG, University of Minnesota, St. Paul, Minn.

*A Race of Crown Rust to Which the Victoria Oat Variety is Susceptible.*¹—The oat variety Victoria (C. I.² No. 2401) recently was reported by the senior author³ as being resistant to 37 physiologic races of crown rust (*Puccinia coronata avenae* Eriks. and Henn.). Since this report was written, the junior writer has found Victoria susceptible to a collection of crown rust made on December 28, 1935, at San Antonio, Texas, by Wallace T. Butler, Agent, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. D. A. Using inoculum obtained from specimens of these infected Victoria plants, the senior writer identified this collection as a race of crown rust new to North America and heretofore not reported from any other place. This new race is hereby designated as key number 41.

The reactions of 11 differential oat varieties (selected in 1931 by the senior writer and by B. Peturson, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada, as standard differential varieties for identification of parasitic races of *Puccinia coronata avenae* occurring in North America) to races 1 to 33, inclusive, have been published by Murphy.⁴ The reactions of these same varieties to races 34, 35, 36, and 37 were determined by the senior writer and published with his permission by Stakman, Levine, Christensen, and Isenbeck.⁵ Peturson⁶ described the reactions of the standard differential varieties to two new races A and B (later designated as standard races 38 and 39, respectively). W. L. Waterhouse, University of Sydney, Australia, has recently identified a new race, designated as number 40, for which the reaction of the differential varieties has not yet been published.

The reactions at approximately 70° F. of the standard differential varieties to race 41 are presented in table 1. The reactions of Victoria and Bond

¹ Journal Paper No. J-355 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 72; and Paper No. 1439 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station, St. Paul, Minn.

² C. I. refers to accession number of the U. S. Dept. Agr., Bur. Plant Industry, Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

³ Murphy, H. C. Reaction of the Victoria oat variety to crown rust. *Phytopath.* 26: 396-397. 1936.

⁴ Murphy, H. C. Physiologic specialization in *Puccinia coronata avenae*. U. S. Dept. Agr. Tech. Bull. 433. 1935.

⁵ Stakman, E., M. N. Levine, J. J. Christensen, and K. Isenbeck. Die Bestimmung physiologischer Rassen pflanzenpathogener Pilze. *Nova Acta Leopoldina* 3: 281-336. 1935.

⁶ Peturson, B. Physiologic specialization in *Puccinia coronata avenae*. *Sci. Agr.* 15: 806-810. 1935.

TABLE 1.—*Host reaction to parasitic race 41 of Puccinia coronata on differential varieties of Avena spp.*

Differential varieties tested		Average infection type produced by race 41
Name	C. I. No.	
Ruakura	2025	1
Green Russian	2890	2
Hawkeye	2464	4
Anthony	2143	4
Sunrise	982	4
Green Mountain	1892	4
White Tartar	551	4
Red Rustproof	1815	3
Sterisel	2891	3
Belar	2760	4
Glabrota	2630	0
Victoria	2401	3
Bond	2733	0

(C. I. No. 2733) also are included in this table because of their outstanding adult resistance to epiphytotics of crown rust when growing under field conditions, and their seedling resistance to a large number of important races. Victoria is known to be resistant in the seedling stage to races 1 to 37, inclusive, and susceptible to race 41. Bond is known to be susceptible only to races 33 and 34. It is probable that additional crown-rust races will be isolated and identified solely on the basis of the differential reaction of either or both Victoria and Bond. For these reasons it seems desirable to include these two differential varieties among the standard varieties used in identifying all collections of crown rust, even though the 41 races discovered to date may be identified by using only the first 11 varieties listed in table 1.

The rare race 41 may not increase in prevalence and distribution and may even disappear, although with an increase in acreage of oat selections, such as described by Stanton *et al.*,⁷ which are endowed with Victoria's resistance to other prevalent races, this new race could increase rapidly. Fortunately, Bond is completely resistant to race 41, thereby making available an immediate source of resistance to it. Hybrid selections combining Bond's resistance to crown rust and to smut with the resistance of certain high-yielding varieties to stem rust have been developed by Murphy, Stanton, and Coffman.⁸ At present, then, for every known parasitic race of

⁷ Stanton, T. R., H. C. Murphy, F. A. Coffman, and H. B. Humphrey. Development of oats resistant to smuts and rusts. *Phytopath.* 24: 165-167. 1934.

⁸ Murphy, H. C., T. R. Stanton, and F. A. Coffman. Hybrid selections of oats resistant to smuts and rusts. *Jour. Amer. Soc. Agron.* 28: 370-373. 1936.

rusts or smuts affecting oats, an oat variety completely resistant to such race is known. So long as this situation exists, breeding for disease resistance in oats has a promising outlook.—H. C. MURPHY and M. N. LEVINE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Iowa and Minnesota Agricultural Experiment Stations, respectively.

THE TWENTY-EIGHTH ANNUAL MEETING OF THE AMERICAN
PHYTOPATHOLOGICAL SOCIETY

ATLANTIC CITY, NEW JERSEY

MONDAY, DEC. 28 TO THURSDAY, DEC. 31, 1936

The Society, as an affiliated organization, joins in the annual meeting of the American Association for the Advancement of Science which is to be held in Atlantic City from December 28, 1936, to January 2, 1937. A.A.A.S. General Headquarters will be at Haddon Hall, Registration Headquarters at the City Auditorium.

The AMBASSADOR HOTEL has been assigned as
SOCIETY HEADQUARTERS

The SESSIONS of the SOCIETY will be held in the
ATLANTIC CITY AUDITORIUM

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ASSOCIATIONS OF MICROORGANISMS IN RELATION TO SEEDLING INJURY ARISING FROM INFECTED SEED¹

J. J. CHRISTENSEN²

(Accepted for publication May 8, 1936)

INTRODUCTION

Recent studies have emphasized the importance of the association of microorganisms in the soil in relation to foot and root rots of cereals. It has been shown that certain saprophytic fungi and bacteria inhibit the development of these diseases.

Henry (6) has demonstrated that seedling injury caused by *Helminthosporium sativum* P. K. and B. and *Fusarium graminearum* Schwabe is reduced by the antibiotic action of the soil microflora. Sanford and Broadfoot (9) found that the microflora of the soil had a deleterious effect on the development of root rot caused by *Ophiobolus graminis* Sacc. Garrett (4) concluded that the differences in soil microflora may account for the variations in prevalence of *Ophiobolus graminis*. Bisby, James, and Timonin (1) showed that *Trichoderma lignorum* (Tode) Harz. suppressed the virulence of *H. sativum* and *F. culmorum* (W. G. Sm.) Sacc. on wheat, and Greaney and Machacek (5) found that *Cephalothecium roseum* Corda also inhibited the pathogenicity of *H. sativum*.

Sanford and Broadfoot (9) and Brömmelhues (2) have shown that under certain conditions saprophytic or antagonistic organisms may actually increase the severity of infection caused by *Ophiobolus graminis*.

Numerous field experiments at St. Paul, Minnesota, proved that seedlings from seed artificially inoculated with, or planted in soil inoculated with, *Fusarium* spp. or *Helminthosporium* spp. seldom were injured. However, when naturally infected seed of wheat and barley was sown there was usually considerable foot and root rot. There is virtually no information on the effect of the microflora of the soil on the development of seedling blight or root rot arising from naturally infected seed. If the soil microflora prevents or appreciably inhibits seedling injury that usually arises from in-

¹ Paper No. 1425 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

² The writer is indebted to Dr. Helen Hart for her assistance and criticism in preparing this paper.

fected seed, it would be of considerable economic importance. It would greatly minimize the importance of treating seed grain infected with foot- and root-rotting organisms. In Minnesota, wheat seed, and particularly barley seed, is often heavily infected with *Fusarium* spp. and *Helminthosporium* spp., the organisms commonly associated with seedling injury (3). Therefore, the effect of the association of microorganisms on the development of seedling blight and root rot resulting from sowing naturally infected barley seed was studied.

METHODS

Most of the organisms used in these tests were isolated from diseased barley seed. Those designated as soil cultures were obtained from soil continuously cropped to barley for 15 years. The soil organisms were obtained by adding about 10 grams of soil to sterile oat hulls in Erlenmeyer flasks, resulting, of course, in very mixed cultures. The organisms, unless otherwise stated, were increased on ground oat hulls to which were added one-half per cent sucrose and usually a small piece of potato. In most cases only young, vigorous cultures were used for inoculation.

Four methods of inoculation were used: (1) dipping or soaking the seed in a suspension of spores and mycelial fragments or extracts of the organisms, (2) pouring the suspension over the seed in the soil, (3) adding the culture of the organisms to soil at, or previous to, the time of planting, and (4) dusting the seed with spores and mycelial fragments. Spore suspensions were strained through cheesecloth or a fine mesh screen to remove pieces of oat hull. Whenever a culture was added to the soil an excess was purposely avoided, and the inoculum was mixed thoroughly with the soil. In the greenhouse tests 2 kinds of soil were used: coarse sand and a mixture of 1 part black loam and 3 parts sand. The soil was autoclaved in shallow pans at about 12 pounds' pressure for from 5 to 7 hours.

Most of the diseased seed was obtained by spraying barley plants growing under muslin tents. Lot F was sprayed with many races of *Fusarium* spp.; lot M with numerous species of fungi other than *Fusarium* spp., mostly *Helminthosporium* spp.; and lot C was not sprayed and was virtually free from root-rotting fungi. Samples of diseased seed, lots A and H, were obtained from farmers.

In order to obviate environmental differences, pots or flats always were randomized and their position usually shifted at intervals of 2 or 3 days. Plots in the field also were randomized.

EXPERIMENTAL RESULTS

Tests with Trichoderma lignorum.—In recent years the writer has made extensive tests on the effect of seed treatment, especially on barley infected

with various Fungi Imperfecti. In a test on varietal response to Ceresan a portion of the seed was dusted with spores and mycelial fragments of *Trichoderma lignorum*. It seemed probable that the lots responding to Ceresan treatment also might respond to the effect of the association with *T. lignorum*, as it is known that the benefit of Ceresan treatment is highly correlated with the severity of seed infection with *Fusarium* and *Helminthosporium*.

Germination tests were made on 2 seed lots, F and M, and plant vigor was noted on 3 lots, C, F, and M. A portion of each seed lot was treated with Ceresan at the rate of 2 oz. per bu., a second portion was dusted with spores and mycelial fragments of *Trichoderma lignorum*, an excessive amount of the fungus material being used, and a third portion remained untreated. Plating tests of seed on nutrient agar at the time of planting indicated that the *T. lignorum* and the pathogens were viable. The grain was sown in 4-foot rows, 100 seeds to the row. Each plot consisted of 3 rows, 1 with Ceresan-treated seed, 1 with *T. lignorum*-dusted seed, another as control. The varieties were replicated several times, and these are listed separately in table 1, while the 54 varieties (including the 5 mentioned varieties) were replicated but once and are considered as a composite group.

According to Weindling (10), the lethal principle of *Trichoderma lignorum* does not accumulate in any considerable quantity, and it is produced most abundantly when the fungus is about 2 days old; consequently, the application of conidia of *T. lignorum* to the seed at time of sowing should be ideal for antibiotic action. It is evident from the data given in table 1 that *T. lignorum* did not increase the average percentage of plants that emerged in the composite group, while Ceresan increased the percentage by 4 per cent for lot F and 10 per cent for lot M. These figures are statistically significant. Lot C did not respond to the Ceresan treatment. Of the five varieties listed in table 1, Peatland, with an average increase in stand of 22 per cent, responded most to Ceresan treatment.

Notes were taken on the relative vigor of plants in the seedling and early boot stages. There was close agreement between the two readings, indicating that the increased vigor persisted beyond seedling stage. Data on plant vigor were taken when plants were about 6 weeks old and indicate that Ceresan caused pronounced increase, while *Trichoderma lignorum* was ineffective (Table 1 and Fig. 1). The average increase in vigor of plants from Ceresan-treated seed was 12, 23, and 0 per cent for lots F, M, and C, respectively, of the 54 varieties.

To determine more accurately the relative vigor of the plants, the green weights of the 5 varieties were obtained. The plants were cut off at the ground line when about 6 weeks old and weighed immediately. Ceresan treatment increased the green weights of plants from seed lots F, M, and C

TABLE 1.—The effect of treatment with *Ceresan* or inoculation with *Trichoderma lignorum* on germination and growth of barley from clean and blighted seed planted in the field^a

Variety	Number of tests			Treatment and percentage of plants emerged										Treatment and percentage increase or decrease in vigor over checks				
				Ceresan			Control			Trichoderma								
	F ^b	M	C	F	M	Av.	F	M	Av.	F	M	Av.	F	M	C	Trichoderma		
																F	M	C
Glabron	10	10	3	41	63	52	33	54	44	39	53	46	+21	+7	+2	+7	+5	0
Lion	8	8	5	14	46	30	15	31	23	13	36	25	-7	+34	0	-3	+6	+7
Manchuria	10	10	5	47	62	55	44	47	46	41	44	43	+10	+22	-1	-4	-2	+2
Minsturdi	10	10	5	36	50	43	27	42	35	27	38	33	+21	+23	-1	+7	-8	+2
Peatland	10	10	5	56	90	73	45	56	51	47	64	56	+21	+25	0	+7	-4	0
Composite of 54 varieties	2	2	1	32	49	41	28	39	34	28	37	33	+12	+23	0	+4	0	+2

^a Results are based on randomized rows each sown with 100 seeds, and the data on vigor of growth were taken on plants six weeks old.

^b Seed lot F was obtained from plants sprayed with *Fusarium* spp. while growing under muslin tents; seed lot M from plants sprayed in a similar manner with numerous species of fungi other than *Fusarium*; and seed lot C was from plants grown in the open and not sprayed.

^c This also includes the five varieties listed in the table.

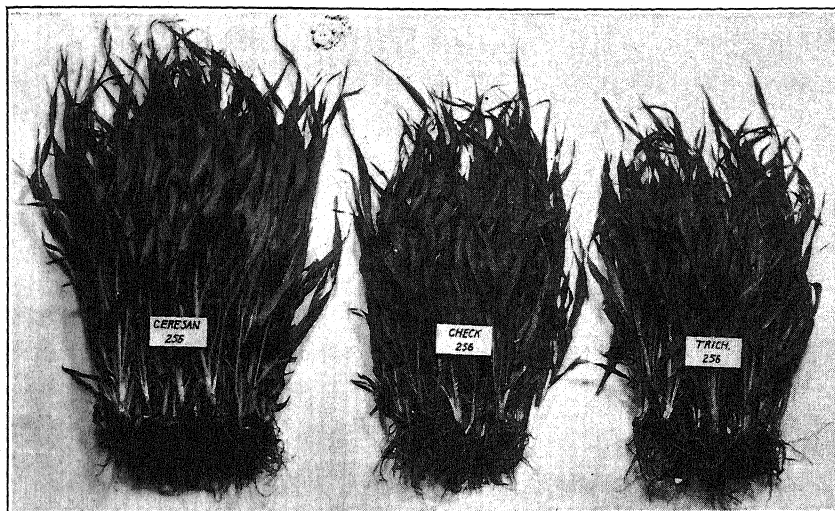


FIG. 1. The effect of treatment with Ceresan and inoculation with *Trichoderma lignorum* on growth of barley from infected seed planted in the field.

by 29, 56, and 0.3 per cent, respectively; similarly, *T. lignorum* increased the green weights by 7, 3, and 1 per cent, respectively. While the figures for *T. lignorum* were not statistically significant, those for the Ceresan lots, with the exception of C, are highly so. The results obtained with Ceresan treatments agree with those of previous tests, namely, that the benefit from seed treatment of barley is approximately proportional to the degree of infection on or in the seed (3).

In view of the negative results with *Trichoderma lignorum* it seemed possible that the soil was infested with microorganisms that inhibited its growth or that *Trichoderma* and other organisms were already present in such abundance that the addition of more *Trichoderma* had no effect. Therefore, experiments were made in sterilized soil in the greenhouse.

In the first test, 2 seed lots of barley, F and H, infected primarily by *Fusarium* spp. and *Helminthosporium* spp., respectively, were used. In addition to the effect of *Trichoderma lignorum*, the effect of a mixed culture of fungi and bacteria obtained from soil was studied. Three different controls were used: (1) sterile oat hulls added to the soil, (2) seed treated with Ceresan, and (3) untreated seed. The sterile oat hulls and the 2 cultures were added to the soil at the time of planting. The results are based on randomized, quadruplicate, 7-inch pots each sown with 25 seeds. Counts were taken on the percentage of seedlings that emerged, the percentage distinctly injured (stunted or deformed), and the green weights when the plants were 1 month old. Henry (7) has shown that high temperatures are conducive

to antibiotic action of certain microorganisms. Consequently, tests were made at relatively high temperatures, which also are conducive to the development of foot and root rot caused by *Fusarium* spp. and *Helminthosporium* spp. In this test, the addition of *T. lignorum* to the soil or the treatment of seed with Ceresan did not materially affect the emergence. *Trichoderma lignorum* did not decrease the number of stunted or deformed plants, but Ceresan did reduce materially seedling injury. This becomes even more evident when one considers the data on green weight. Ceresan increased the green weight of lots F and H 31 and 23 per cent, respectively. In lot F, *T. lignorum* decreased the green weight by 11 per cent, and in lot H increased it 3 per cent. These differences probably are not significant.

The results in table 2 indicate that the addition of sterile oat hulls to the soil causes seedling injury and decreases the vigor of the plants. Sallans (8) found that wheat seedlings were severely injured if sterilized oat hulls were added to nonsterilized soil but not if added to sterilized soil. He attributed this injury not to the toxic substances in the medium but to the growth of the soil-inhabiting organisms upon the sterile material. In the present study it seems likely that the parasites on the seed spread to the oat hulls in sterilized soil, grew rapidly, and attacked the young seedlings.

TABLE 2. *The effect of various treatments on germination and growth of barley from blighted seed planted in sterilized soil^a*

Treatment of sterilized soil	Percentage of plants				Green weight (in grams)	
	Emerged		Stunted or deformed			
	F ^b	H	F	H	F	H
None—control	61	76	21	14	5.4	9.8
Sterile oat hulls added	58	76	33	26	4.8	7.7
<i>Trichoderma lignorum</i> added	64	81	21	15	4.8	10.1
Mass culture (from soil) added	59	80	25	19	5.4	8.1
None—seed treated with Ceresan	63	76	8	7	7.1	12.1

^a Results are based on randomized quadruplicate pots each sown with 25 seeds.

^b F and H were infected chiefly with *Fusarium* spp. and *Helminthosporium* spp., respectively.

A similar experiment was made on sterilized and nonsterilized soil, using the same seed lots, F and H, and also another lot, A, which was badly discolored by fungi, chiefly *Alternaria* spp. These results are summarized in table 3. As in previous tests, the addition of *Trichoderma lignorum* cultures to sterilized and nonsterilized soil did not increase germination, seedling vigor, or green weight. However, the percentage of seedlings stunted or deformed in seed lots F and H were somewhat reduced by the addition of

T. lignorum to both the sterilized and nonsterilized soil. Seed lot A developed virtually no seedling blight.

It is possible that the race of *Trichoderma lignorum* used was not particularly antibiotic to fungi on the seed, but the following tests proved, on the contrary, that it was distinctly antibiotic toward four races of *Helminthosporium sativum* isolated from seed. Clean seed of barley was inoculated by dipping it in a heavy spore suspension of *H. sativum* or one of *H. sativum* and *T. lignorum*. One portion was sown in sterile soil and another in sterile soil that had been inoculated 24 hours previously with a spore suspension of *T. lignorum*. Pots were randomized and sown in duplicate, each with 50 seeds. The results are given in table 4 and are in accord with those of other workers. There were better stands and fewer stunted or deformed seedlings when the seed was inoculated with a spore suspension of *H. sativum* and *T. lignorum* or when it was sown in soil to which *T. lignorum* was added. Plants of these lots also were more vigorous than those of the control that had been inoculated with *H. sativum* alone. *Trichoderma* was distinctly antibiotic to *H. sativum*.

The antibiotic effect of *Trichoderma lignorum* on several species of *Helminthosporium* was tested in other experiments, and the results were similar. There were indications that the less virulent the race or species of *Helminthosporium* the more pronounced was the effect of *T. lignorum*.

Races of *Trichoderma lignorum* may differ in antibiotic effect, however, for a race given to the writer by I. Tervet³ was less antibiotic toward *H. sativum* than the one used in this work. Differences in antibiotic action among races of *T. lignorum* was noted by Weindling (10).

Tests with other organisms.—A number of investigators have shown that several organisms besides *Trichoderma lignorum* may inhibit the development of root rot. Moreover, there is sometimes a high degree of specificity in antibiotic action among fungi. Therefore, experiments were made on the antibiotic effect of several microorganisms isolated either from the seed or soil. These tests were made in a manner similar to those described for *T. lignorum*.

As with *T. lignorum*, the most extensive tests were made in the field. In one test 3 seed lots from 2 varieties of barley were used, and 100 seeds of each lot were sown in randomized quadruplicate rows. Severely infected seed lots were planted so that the effect of the association of microorganisms could be more easily ascertained. At the time of planting, the seeds were immersed in heavy suspensions of spores and mycelial fragments of the respective organisms. Notes were taken on percentage stand and vigor of plants, and at the end of the experiment the green weights were determined. The data in table 5 indicate that none of the 12 organisms used influenced

³ Commonwealth Fellow 1933-1934, University Farm, St. Paul, Minnesota.

TABLE 4.—*The effect of Trichoderma lignorum on germination and growth of barley from clean seed inoculated with four races of Helminthosporium sativum planted in sterilized soil*

Races of <i>H. sativum</i>	Percentages of plants ^a								
	Emerged			Stunted			Contorted leaves		
	H ^b	H + T	H + S I	H	H + T	H + S I	H	H + T	H + S I
21	84	94	94	46	12	6	52	32	15
22	88	94	98	33	8	6	57	27	14
23	86	88	96	25	17	8	78	31	21
24	88	98	94	10	4	3	17	15	10

^a Results are based on randomized duplicate pots each sown with 50 seeds.

^b H = Seed inoculated with a spore suspension of *Helminthosporium sativum*.

H + T = Seed inoculated with a spore suspension of *Helminthosporium sativum* in combination with *Trichoderma lignorum*.

H + S I = Seed inoculated with a spore suspension of *Helminthosporium sativum* and planted in soil inoculated with *Trichoderma lignorum*.

the stand or green weight. On several occasions notes on the relative vigor of the plants were taken, but there were never any indications that the organisms increased or decreased the vigor of the plants; in this respect, however, the effect of the Ceresan treatment was very striking.

A similar test also was made with 5 organisms, using 2 lots of diseased barley from 5 varieties: Glabron, Manchuria, Minsturdi, Peatland, and Wisconsin No. 38. Each lot was sown in duplicate randomized rows, 100 seeds to a row. The data are summarized in table 6. Although considerable seedling injury occurred, there was no beneficial effect from the addition of 5 microorganisms. It is noteworthy that the amount of seedling blight arising from naturally infected seed was not increased by the addition of *Fusarium culmorum* to the seed at time of planting. Apparently the antibiotic organisms in the soil inhibited *F. culmorum* when seed was artificially inoculated but were ineffective when seed was naturally infected.

In still another test large quantities of all the organisms mentioned in table 5, with the exception of *Fusarium culmorum*, were applied to the soil. Three diseased lots of barley seed, comprising 2 varieties of barley, were sown in open furrows to which was added about 500 cc. of oat-hull cultures. Each seed lot was replicated 4 times. Considerable seedling blight developed. The addition of the large quantities of the mixed cultures at seed level did not prevent seedling injury, while again Ceresan was decidedly effective.

The inhibiting effect of several microorganisms on seedling blight arising from infected seed also was studied in the greenhouse. Minsturdi seed infected naturally with *Fusarium* spp. and *Helminthosporium* spp. and also

TABLE 5.—The effect on germination and growth of barley from blighted seed inoculated with twelve organisms and planted in the field^a

Inoculum	Stand in percentage				Green weight (in grams)			
	Minsturdi		Minn. No. 462	Average of 3 lots	Minsturdi		Minn. No. 462	Average of 3 lots
	F ^b	M	F		F	M	F	
Control—A	31	39	35	35	145	214	203	187
Bacterium	22	41	42	35	114	195	250	186
<i>Actinomyces scabies</i>	29	46	38	38	115	214	232	187
<i>Aspergillus clavatus</i>	32	45	42	40	154	239	233	209
<i>Aspergillus giganteus</i>	30	40	35	35	147	210	194	184
<i>Aspergillus terreus</i>	30	43	45	39	136	256	255	216
<i>Cephalothecium roseum</i>	30	47	32	36	137	225	253	205
<i>Chaetomium spirochaete</i>	31	39	38	36	211	215	233	220
<i>Cunninghamella</i> sp.	31	40	30	34	132	275	188	198
<i>Fusarium culmorum</i>	37	43	33	38	185	223	237	215
<i>Penicillium expansum</i>	33	44	36	38	116	226	235	192
<i>Penicillium martensii</i>	30	41	36	36	168	202	199	190
<i>Trichoderma lignorum</i>	33	48	39	40	163	241	219	208
Mixture of above organisms ^c	34	52	41	42	145	281	233	220
Control—B	34	43	41	39	161	248	194	201

^a Results are based on randomized quadruplicate rows each sown with 100 seeds.

^b Seed lot F was obtained from plants sprayed with *Fusarium* spp. while growing under muslin tents, and seed lot M was from plants sprayed in a similar manner with numerous species of fungi other than *Fusarium*.

^c Except *Fusarium culmorum*.

TABLE 6.—The effect on germination and growth of barley^a from blighted seed inoculated with five organisms and planted in the field

Inoculum	Percentage of plants emerged		Green weight			
			In grams		Percentage increase or decrease over control	
	F	M	F	M	F	M
Bacterium	31	34	231	209	-15	-13
<i>Actinomyces scabies</i>	32	37	241	241	-11	+1
<i>Cephalothecium roseum</i>	31	36	258	227	-5	-5
<i>Fusarium culmorum</i>	31	33	248	231	-9	-3
<i>Penicillium expansum</i>	34	35	276	246	+1	+3
Control	35	33	272	239	-	-

^a Results are based on the averages of 5 varieties (Glabron, Manchuria, Wis., No. 38, Minsturdi, and Peatland), each grown in randomized duplicate plots.

Minn. No. 462 infected with *Fusarium* spp. were sown in flats containing sterilized sand. The cultures of 12 organisms were added separately to sand in flats 2 days before planting. The seed was sown in randomized duplicate rows, 50 seeds in each row. The results are summarized in table 7. As in the field tests, the addition of microorganisms to the soil was ineffective in reducing seedling injury.

TABLE 7.—The effect on germination and growth of barley from blighted seed planted on sterilized soil inoculated with twelve organisms forty-eight hours before planting

Inoculum	Percentages of plants ^a							
	Emerged				Stunted or deformed			
	Minsturdi		Minn. No. 462	Average of 3 lots	Minsturdi		Minn. No. 462	Average of 3 lots
	F	M	F		F	M	F	
Bacterium	54	93	77	75	18	7	14	13
<i>Actinomyces scabies</i>	52	85	77	71	20	8	14	14
<i>Aspergillus clavatus</i>	57	84	81	74	10	12	12	11
<i>Aspergillus giganteus</i>	56	89	80	75	16	12	19	16
<i>Aspergillus nidulans</i>	51	86	78	72	28	9	13	17
<i>Cephalothecium roseum</i>	60	83	72	72	25	12	19	19
<i>Chaetomium spirochaete</i>	64	87	80	77	12	12	12	12
<i>Cunninghamella</i> sp.	63	87	74	75	14	12	18	15
<i>Penicillium elongatum</i>	63	93	73	76	17	11	24	17
<i>Penicillium expansum</i>	61	88	19	13
<i>Penicillium martensii</i>	91	68	12	19
<i>Trichoderma lignorum</i>	50	89	80	73	16	11	18	15
Sterile oat hulls	49	87	79	72	20	11	13	15
Control	61	89	81	77	18	6	20	15

^a Results are based on randomized duplicate rows each sown with 50 seeds.

Sanford and Broadfoot (9) found that filtrates from certain organisms gave greater protection than the living cultures. In order to test the efficacy of extracts of microorganisms, diseased grain of Peatland barley was immersed for 4 hours in a water extract of 5 organisms. The extract was made by chopping up the culture and soaking it in sterilized water for 24 hours. This was then strained through cheesecloth; hence the extract contained also viable spores and fragments of mycelium, or bacteria. The seed was sown in randomized quadruplicate rows, 50 seeds in each. The data are presented in table 8. *Actinomyces scabies* (Thaxt.) Güss. and *Trichoderma lignorum* apparently decreased the number of diseased plants in lot F infected with *Fusarium* but not in lot M infected primarily with *Helminthosporium*. In fact, *Trichoderma* appears to be injurious to this lot, as the percentage of

TABLE 8.—The effect on germination and growth of Peatland barley from blighted seed soaked four hours in an extract of five different organisms and planted in sterilized soil

Inoculum	Percentages ^a					
	Stand		Stunted		Disease-free	
	F ^b	M	F	M	F	M
Bacterium	56	95	29	33	28	9
<i>Actinomyces scabies</i>	52	95	21	27	45	11
<i>Aspergillus nidulans</i>	56	94	26	23	35	16
<i>Cephalothecium roseum</i>	56	94	27	31	36	10
<i>Trichoderma lignorum</i>	55	86	20	27	45	19
Control	60	96	26	24	34	16

^a Results are based on randomized quadruplicate rows, each sown with 50 seeds.

^b Seed lot F was obtained from plants sprayed with *Fusarium* spp. while growing under muslin tents, and seed lot M was from plants sprayed in a similar manner with numerous species of fungi other than *Fusarium*.

plants that emerged was decreased 10 per cent. Perhaps if higher concentration of the extracts had been tried or if the period of immersion had been increased, the results might have been different.

The antibiotic action of several organisms to *Helminthosporium sativum* in sterilized soil was proved by inoculating virtually disease-free barley seed with a mixture of a spore suspension of *H. sativum* and several other organisms. The results are given in table 9 and are based on randomized quadruplicate pots each sown with 25 seeds. All of these organisms increased the number of plants that emerged, although some more than others.

TABLE 9.—The effect on germination and growth of barley from clean seed inoculated with *Helminthosporium sativum* in combination with six other organisms and planted in sterilized soil

Inoculum added to <i>H. sativum</i>	Percentage of plants ^a	
	Stand	Stunted or deformed
None— <i>H. sativum</i> only	80	36
<i>Bacterium</i>	83	17
<i>Basisporium gallarum</i>	89	25
<i>Cephalothecium roseum</i>	88	31
<i>Chaetomium spirochaete</i>	88	14
<i>Penicillium</i> sp.	91	31
<i>Trichoderma lignorum</i>	81	25
Control—uninoculated	97	3

^a Results are based on randomized quadruplicate pots each sown with 25 seeds.

All of them reduced the percentage of stunted seedlings; *Chaetomium spirochaete* Palliser and the bacterium were especially effective.

Apparently, there are a number of organisms that have antibiotic effect on *Helminthosporium sativum* on the seed or in the soil. If, however, *H. sativum* and other fungi are within the seed coat or have invaded the tissues of the seedling, the antibiotic organisms have little or no effect.

DISCUSSION

The results of these experiments indicate that the microflora of the soil at University Farm, St. Paul, did not prevent or appreciably decrease seedling blight or root rot arising from infected barley seed. Preliminary tests with infected wheat and oats gave similar results. The behavior of infected seed lots was quite similar whether sown in the field or greenhouse, in sterilized or nonsterilized soil. Under all conditions studied, diseased seed always germinated poorly or weakly and developed considerable seedling blight and foot and root rot, while clean seed usually developed healthy plants. The fact that treatment of barley seed is generally beneficial in proportion to the extent of seed infection also is an indication that the primary source of seedling injury often is infected seed. This may not be true under certain cultural practices, as sowing barley on disced cornstalk land. In certain years it is not uncommon to have barley grain with 10 to 25 per cent or more infection by *Fusarium*, *Helminthosporium*, and other root-rotting organisms. Naturally, the sowing of seed infected with virulent parasites is not uncommon. It is quite possible, however, when barley seed is only lightly infected, that the soil microflora may suppress and even prevent seedling injury. This certainly is true when the pathogen is applied directly to the seed or to the soil. Obviously, the soil microflora plays an important rôle in suppressing or eliminating soil-borne parasites, and the importance of proper rotation should not be minimized.

The results obtained are of considerable practical significance. They help to explain, at least in part, why considerable *Fusarium* and *Helminthosporium* seedling injury may sometimes develop when good rotation is practiced and even on summer-fallowed land. They emphasize the necessity of treating seed, so as to eliminate insofar as possible the pathogen on or in the seed. They also point out the importance of sowing disease-free barley seed when studying the effect of various cropping systems on the development of root rot. It is important to remember that, although seed treatment will eliminate much of the primary infection, it will not prevent secondary infection. Eventually, all barley and wheat plants, at least under Minnesota conditions, become more or less attacked by soil-borne parasites, but the greatest injury from *Fusarium* and *Helminthosporium* usually results from early seedling infection. Although most of the seed treatment tests were made

with ordinary Ceresan, additional tests with New Improved Ceresan at the rate of one-half ounce per bushel have given equally good results.

SUMMARY

The results indicate that the soil microflora does not have a marked effect on seedling blight arising from infected barley seed. There were no differences in germination or amount of seedling injury when barley seed naturally infected with *Helminthosporium* or *Fusarium* was planted in sterilized and nonsterilized soil.

Antibiosis is perhaps of little importance in suppressing seedling injury from diseased barley seed. The addition of *Trichoderma lignorum* and several other fungi and bacteria to naturally infected barley seed or to sterilized or nonsterilized soil did not inhibit or delay the parasitic action of seed-borne parasites. Soaking infected seed in an extract of these organisms also gave negative results.

The addition of *Trichoderma lignorum* and certain other fungi and bacteria to seed or sterilized soil inoculated with *Helminthosporium sativum*, however, increased the stand, decreased the number of deformed and stunted plants, and suppressed seedling injury.

Naturally infected barley seed is one of the primary sources of seedling injury in Minnesota and should not be sown unless properly treated.

Treating naturally infected seed with Ceresan improved the stand, decreased seedling injury, and materially increased plant vigor. Preliminary tests with New Improved Ceresan also gave good results.

UNIVERSITY FARM,
ST. PAUL, MINN.

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COPPER DEFICIENCY IN SUGAR BEETS

D. A. VAN SCHREVEN¹

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INTRODUCTION

There are various practical examples of surplus yields obtained by the application of copper combinations. In part of the instances certain symptoms of disease even disappeared, as in the case of the so-called "reclamation disease" in Holland. This disease occurs in the Netherlands chiefly in the provinces Drente, Overijssel, Utrecht, Gelderland, Noord-Brabant and Limburg, and especially on heath land, which is very wet in the winter, or was so before the drainage, and dries up in the summer. On sandy soils, in cultivation for a few decades only, the disease appears to occur more frequently than on those longer in cultivation. It occurs also on peaty soils; particularly, on those not much used for peat cutting and of which the peat bog has more the character of lowland (moor) fen than of upland fen.

In Germany the disease is called "Heidemoorkrankheit" or "Urbar-machungskrankheit" (32, 33) or "Weisseuche" (4); in England it is indicated as "Reclamation disease" or "Heath bog disease," also "Yellow tip" (17).

This disease was described for the first time by Elema (5) as occurring in Holland in oats on reclaimed heath land. Barley also appeared to be very sensitive.

Meijer and Hudig (27) state as regards oats that, initially, the plants grow normally. However, as soon as they have formed a few leaves, yellow grayish spots suddenly turn up in the field, especially during warm, dry weather. The following day the leaves appear to have yellow whitish arid points in the ill spots. One or two days thereafter these points have become larger and are, especially when viewed from a distance, almost white. These symptoms are not always the same; sometimes the arid points are a bright white or are a little more yellow; there are cases in which the symptoms only appear at a later stage of growth. It occurs that the plants are more yellow or greener or that the leaves are striped yellow green and yellow. The plants recover a little during rainfall. Sometimes they die completely and in other instances they still form panicles containing empty or very light grains; the chaff is often white. The culms form new

¹ Phytopathologist of the Instituut voor Suikerbietenteelt, at Bergen op Zoom (The Netherlands).

a dirty green and becomes finally brownish. The upper internodes of the culms are much too short but their leaves are normal. In cases of milder affection the plants grow normally until they shoot—sometimes even particularly well—but then they do not make further headway. The upper internodes of the culm do not develop. The ripening takes place in the same way as in the more serious cases. The grains are very light or empty. The stubble sends up secondary shoots. The disease could be controlled by 80,000 kg. of compost per hectare (27) or by copper sulphate (13, 14, 15).

Meijer and Hudig (27) intimated that the cause might be found in a kind of black moor or black heath bog, locally called “pik” or “gliede.”

Smith (41) attributed the disease to an organic material with slightly acid qualities, which he termed “gliedine”.

Only the investigations of Brandenburg (2) showed that the disease is simply the result of copper deficiency. In the absence of copper, symptoms were obtained in water cultures with oats that were exactly the same as those of the “reclamation disease” occurring in practice.

Sjollesma (40) along other channels came to the same conclusion. In regions where the “reclamation disease” occurred, cattle were often found to suffer from the “licking disease”, which is accompanied by anaemia. The symptoms are: considerable backwardness in development, marked emaciation, dryness of the hair, inclination to lick, to bite wood, etc., lack of appetite, consumption of extraordinary food, refusal of grass and, therefore, failure to graze normally, a muzzy, dreamy expression of the eyes. A cure can be quickly effected by means of copper sulphate. This implies that cattle on farms where the reclamation disease is prevalent absorb too little copper. Analyses of compost showed a copper content of 190–400 mg. per kilo, i.e., when applying 80,000 kg. compost there is an amendment of 15.2–32 kg. of copper, which quantity corresponds to 61–130 kg. copper sulphate.

Meijer and Hudig (27) state that oats are most sensitive to this disease, followed by rye and black President oats; while potatoes, lupines, and serradella appear to be rather insensitive. Grassland loses the good grasses and retains chiefly *Holcus lanatus* and white clover. On very “sick” soil, potatoes and grass will not grow well, either.

On basis of field experiments of several years with various crops Meijer (28) arrives at the following conclusions:

- 1) The quantity of copper sulphate to be employed has relation with the humus content of the soil. In case of humus-rich soils it is safe to apply 100 kg. per ha (sulphate of copper testing 25 per cent Cu). Already in case of 7 per cent humus this may be necessary.

- 2) 50 kg. copper sulphate per ha is not always conclusive, but quantities of more than 100 kg. do not appear to be necessary.
- 3) Excess of copper sulphate may sometimes do harm; 50 kg. per ha may already be too much for sandy soils without humus.
- 4) Liming aggravates the reclamation disease.
- 5) An application of copper sulphate, efficient in relation to the humus content, (50 to 100 kg. per ha) is probably sufficient for at least 7 years.

In an experiment carried out at Drouwerveen in 1936 on a soil containing about 17 per cent humus and affected by the reclamation disease, a quantity of 50 to 150 kg. copper sulphate appeared to increase the crop of beets by about 44 per cent and the sugar yield by 60 per cent. 50 kg. per ha was adequate. The plants in the untreated fields seemed to have during the first $1\frac{1}{2}$ months after coming up a lighter color than those dressed with copper sulphate. A month later the color of the leaves was normal and again 2 to 3 months later the foliage had a slightly greener color and was moreover shorter and steeper than that of the plants with copper sulphate. The growth of the beets of the untreated plots remained from the outset behind those of the treated fields. This trial field received shortly before the application of the seeds a quantity of farmyard manure of 25 tons per ha. It is not impossible that copper was added simultaneously with the farmyard manure and that consequently symptoms of disease in the plants without copper sulphate did not occur distinctly.

In the summer of 1935 symptoms of copper deficiency were studied in water cultures with sugar beets (38, 39).

MATERIALS AND METHOD

Chemicals "Kahlbaum pro Analyse" previously twice recrystallized in double-distilled water, were employed for the water cultures. The water was distilled from stills of pyrex glass and also received in stills of pyrex glass. Also double distilled water was used for the solutions, whilst pyrex tumblers of 1 liter with paraffined plaster covers served as pots. In each pot one beet plant was grown.

The nutrient solution contained per liter: 1.00 g. nitrate of potash, 0.50 g. sulphate of magnesia, 0.50 g. sulphate of calcium, 0.70 g. phosphate of calcium (tertiary), and furthermore; 60 mg. ferro ammonium sulphate, 5 mg. sulphate of manganese, 0.7 mg. boric acid, 0.5 mg. sulphate of zinc.

The tertiary phosphate of calcium was obtained by conversion of phosphate of sodium and chloride of calcium, which also were previously twice of sodium and chloride of calcium, which also were previously twice crystallized. Ten pots received 0.5 mg. CuSO_4 5aq., while 10 control pots received no copper. To make sure that the solutions to which no copper was applied were indeed free of copper, an oat plant (variety "Zege")

was grown beside each beet plant, as it is known that oats are very sensitive to copper deficiency and show very characteristic symptoms.

The seed of the beets (variety Hilleshög) was germinated before in good leaf-mould and transferred to the pots on June 26 after the formation of the first 2 heart leaves. Plants of equal size were selected and the roots carefully cleaned with double-distilled water.

The oat grains were kept for a week on filter paper in dishes and were kept moist by copper-free water. Three days after the transfer of the young beet plants they were placed in the solutions. The solutions were renewed every 4 weeks and regularly supplemented.

RESULTS

With the aid of the oat plants, it could be shown that the solutions, to which no copper was applied, were indeed free of copper, for, as noted by Brandenburg (3), the first very typical symptoms of the disease appear in oats after the lapse of 4 weeks.

The beets without copper reacted already after 19 days very distinctly with a light chlorosis which increased gradually in intensity. This chlorosis began in most cases at the top of the leaves and spread fairly regularly over the whole leaf surface resulting in a marbled appearance. The veins contrasted as green against the light yellow greenish interstitial leaf tissue. The symptoms were clearest in the oldest leaves and those at half the height. In some cases the lower leaves were free of chlorosis, while the taller leaves were clearly marbled (Fig. 1, A). No chlorosis developed in the heart leaves. The green parts of the leaves showed sometimes a blue-greenish tint; in other cases the color was normal, *viz.*, dark green.

In the chlorotic parts of the older leaves the leaf tissue died after 2 months and became a gray brownish to gray or white. The chlorotic leaves were mostly a little thinner than the leaves of the healthy control plants and were sometimes a little arched marginally (Fig. 2, A). The leaves of the diseased plants were, during the first 6 weeks, in general, somewhat larger than the corresponding leaves of the control plants. Furthermore, the habit was on the whole rather normal. They produced only very little or no starch, which was demonstrated by treating leaves collected at 5 p.m. on July 26 (one month after planting) a few diseased and healthy leaves according to Sachs' iodine experiment, in which case the starch of the leaves becomes colored by the iodine, present in the solution of potassium iodine following removal of the chlorophyll by boiling with concentrated alcohol. The greatest accumulation of starch was observable in the veins of the diseased leaves, whilst the rest of the leaf tissue became slightly brown or remained unchanged as to color in contrast with the blue black discoloration of the leaf tissue of the healthy plants (Fig. 2, B). After a few weeks this

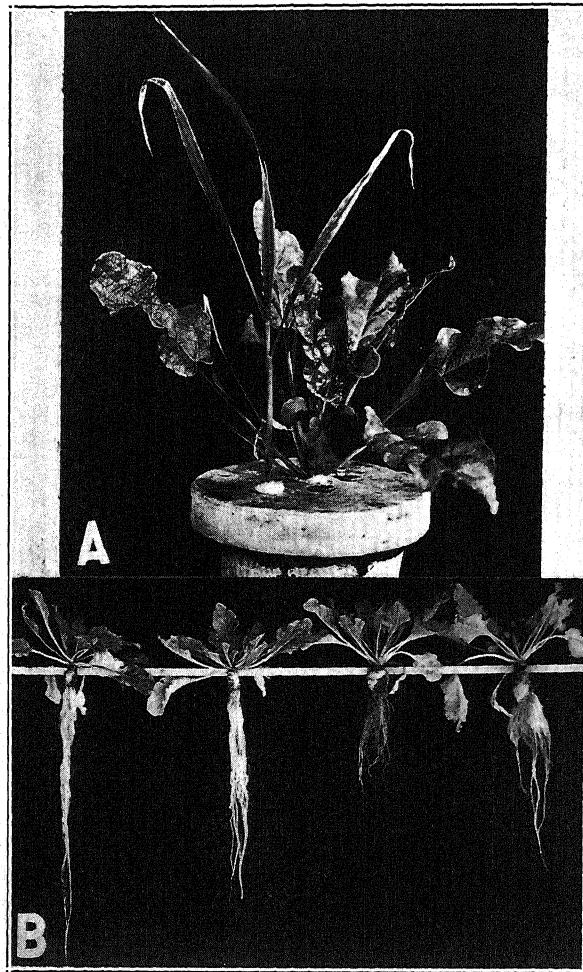


FIG. 1. A. Symptoms of copper deficiency in oats (variety "Zege") and in sugar beet (variety Hilleshög). The oats plant shows the white points in the leaves which are characteristic for the reclamation disease. The lower and the heart leaves of the sugar beet do not show chlorosis, but the others show marbling. B. The two beet plants on the left did not receive copper; those on the right received 0.5 mg. copper sulphate per liter. A distinct difference is noticeable between the root systems of the two series. Where no copper was applied the tap root remained very small, and the side roots became long and thin.

test for starch was repeated, with the same result. The heart leaves produced relatively the highest amount of starch in the diseased plants, though also distinctly less than in the sound healthy ones.

It may be concluded from this that copper is indispensable for beets and plays an essential role in the function of the chlorophyll, as is probably the

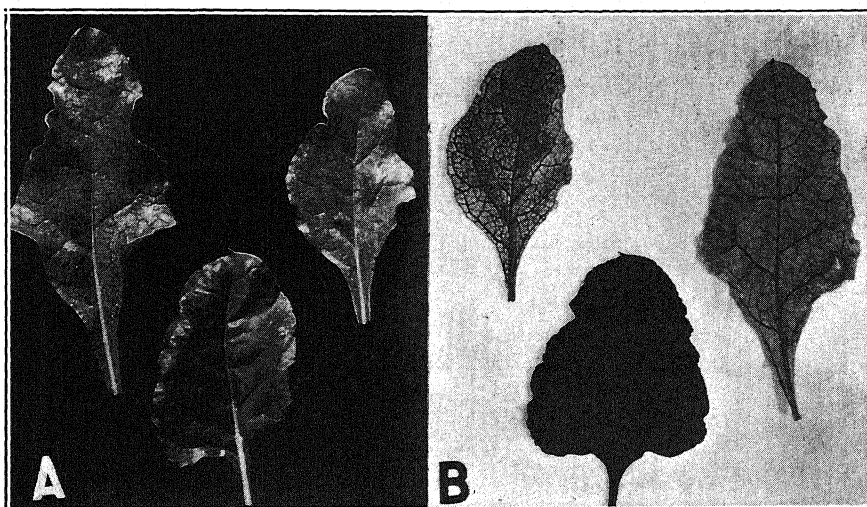


FIG. 2. A. Both upper leaves originated on two beet plants to which no copper was applied. They show the finely shaded chlorosis and have locally arched edges. The other leaves originated on a plant dressed with 0.5 mg. copper sulphate per liter. B. The same leaves after treatment according to Sachs' iodine experiment at 5 p. m. In the two upper leaves only the leaf veins became dark colored but the other leaf became blue-black over its whole surface.

case with the haemoglobin in bodies of animals (7, 8, 9, 10, 11, 18, 19, 20, 21, 22, 26, 29, 41).

In the absence of copper the lateral roots were generally longer and thinner than those of the control plants and pure white (Fig. 1, B). The tap root remained much retarded in development, compared with the tap roots of the sound plants, though the latter disposed, during part of the vegetation period, of relatively less food than the corresponding diseased plants, because the healthy oats growing beside the beets, probably had absorbed more food from the solution than the corresponding plants that received no copper.

To obtain a better comparative picture, the oat plants were removed on August 12. The diseased plants then had an average height of about 25 cm. and the healthy ones of about 45 cm. The beets were harvested on September 11 and the yields are presented in table 1. The great significance of the element copper for the beet appears clearly from the figures. In the first place the large difference in weight of roots is striking. The weight of the roots of the diseased plants is, on an average, 10.522 ± 0.47 grams and of the sound ones 30.597 ± 1.4 grams. The difference amounts to 20.075 ± 1.48 grams and is, therefore, reliable.

TABLE 1.—*Effect of copper on sugar beets in 1935. Duration of experiment, 71 days*

	No. pot	Weight of roots	Average weight of roots	Weight of foliage	Average weight of foliage	Average sugar content	Average weight of sugar	Dry matter content roots	Ash content roots
		grams	grams	grams	grams	grams	grams	grams	grams
No copper	1	10.880		15.780					
	2	11.650		32.050					
	3	12.175		29.825					
	4	13.100		30.510					
	5	10.760		25.175					
	6	9.605	10.522	19.590	24.411	15.2	1.60	24.73	1.050
	7	9.885	± 0.47	24.600	± 1.69				
	8	9.910		25.825					
	9	8.250		20.250					
	10	9.005		20.505					
0.5 mg. CuSO_4 5 aq. p. L.	11	27.800		25.720					
	12	26.010		24.020					
	13	24.960		24.970					
	14	33.750		26.720					
	15	37.275		24.600					
	16	35.525	30.597	28.005	25.458	16.85	5.16	24.70	0.924
	17	35.725	± 1.47	25.620	± 0.419				
	18	30.635		24.870					
	19	25.525		23.625					
	20	28.760		26.430					

The relation of weight of foliage to weight of roots is, for the diseased plants, 2.32 and for the sound ones 0.83; therefore, in the first case, it is considerably larger than in the second one at this stage of growth.

The average sugar content of the diseased plants is 15.2 per cent and of the sound ones 16.85 per cent, so that the weights of sugar per beet are 1.60 and 5.16 grams, respectively. The plants that received copper, therefore, produced, after 71 days, an average of 3.2 times the quantity of sugar produced by the plants to which no copper was applied. The average weight of foliage of the 2 series does not differ much. But the difference would, no doubt, have been larger—also the difference in the sugar yield—had the experimental period been prolonged, because a few weeks before the harvest no noticeable growth could any longer be ascertained in the diseased plants. This was also the reason why the trial was terminated on September 11. The dry-matter and ash contents differ but little in the two series.

DISCUSSION

In the periodical "Mededeelingen" of the "Instituut voor Suikerbietenteelt" (Bergen of Zoom, Holland) (37), issue of January, 1936, the writer dealt with the principal diseases that, in Europe, cause yellow discoloration or symptoms of chlorosis in beets, *viz.*: Nitrogen deficiency; manganese deficiency; black wood-vessel disease, caused by *Pythium* sp.; verticilliosis or fusariosis; mosaic disease; and yellowing disease, to which should now possibly be added copper deficiency.

When comparing the symptoms of copper deficiency in beets with those of the *Pythium*-disease, described by Brandenburg (2), and called black wood-vessel disease by Quanjér (32), there is similarity insofar as in both cases a light yellow greenish discoloration is found between the veins of the outer leaves. Early in the incipency of the disease the marbling is in both cases very finely shaded. The ramifications of the veins contrast as a fine network against the light-colored tissue. In the "Black wood-vessel disease," this chlorosis is mostly followed by withering of the outer leaves, which was not observed in the plants suffering from copper deficiency. For these plants the transport of water to the leaves can take place without disturbance, as the wood vessels in the roots do not degenerate nor do they assume a dark color, as in the case of the black wood-vessel disease. In water cultures the supply of water to the plant is, however, particularly ideal, and it is evident that copper-deficient beets in the field show symptoms of earlier withering, owing to severe drought, than do sound beets, particularly if it also appears in the field that the relation of weight of foliage to weight of roots is larger for diseased plants than for sound ones.

Furthermore, it is quite possible, even probable, that plants with a root system weakened by copper deficiency, which is also long and thin, are much more sensitive to *Pythium* and other root parasites than plants that dispose of sufficient elements.

In the water cultures the dead leaf tissue showed finally a brown greyish, greyish, sometimes white, color. It is not unlikely that under natural conditions, whereby the plants are exposed to rain, the dead parts may finally become dark brown and (or) black in reacting to microorganisms, as is the case of the yellowing disease (32). Thorough field observations should reveal in what respects the symptoms of copper deficiency differ in practice from those induced in water culture.

The provisional impression is, on basis of symptoms of copper deficiency in water cultures compared with manganese-deficiency—simultaneously created in water cultures—that the principal points of difference between the two diseases are the following:—

- (1) When there is copper deficiency the shading of the marbling is finer than in the case of manganese deficiency.
- (2) In case of manganese deficiency the plants, in general, impress one as being more yellow.
- (3) In case of manganese deficiency the leaf margins of the diseased leaves are generally more strongly curled in an upward direction.

As regards copper deficiency in other plants, it can be stated that Wilson and Townsend (47) described a chlorosis in lettuce that could be corrected by the application of copper salts. The disease occurred with a pH of 4.8.

Disease symptoms in fruit trees that could be corrected by application of copper sulphate have frequently been described as "exanthema" or "die back." The disease is especially known to affect citrus in Florida, as early as 1875. Since then this disease has been found in California, Cuba, Puerto Rico, Yucatan, Dutch Guinea, Brazil, Hawaii, Italy, Australia, the Philippines, and South Africa. A chlorosis in fruit trees caused by copper deficiency in South Africa was described by Anderssen (1) and Isaac (16). Anderssen states that the disease occurs on light sandy soils with a pH of 5.5-6.5. Plums, peaches, apricots, apples and pears react with chlorotic symptoms and death of the leaves and tips of the shoots. Isaac states that the lower leaves especially are affected and that application of calcium promotes the disease. *Vide* for further literature, Jacks and Scherbatoff (17). The trees may be cured by (a) application of copper salts to the soil in the neighbourhood of the bole, (b) spraying the trees with Bordeaux mixture and (c) also by putting copper salts into the bole.

Though copper deficiency may occur on alkaline soils, this disease is mostly found to occur on sour soils; this in contrast with manganese and iron deficiency. Also, boron deficiency occurs mostly (however, not always) on soils with an alkaline reaction. Liming, however, promotes copper deficiency in most cases; the same applies to manganese, iron and boron deficiency.

These four deficiency diseases will, moreover, when lime is applied, appear to be more pronounced in plants that grow best with an alkaline reaction. Firstly, these elements will be converted in such a soil condition into a less good soluble form; and secondly, relatively more of these elements will be necessary at a certain stage of growth, owing to the stronger growth of the plant caused by the high pH, than would be the case under a lower pH. This applies, therefore, particularly, to beets, peas, and beans.

Experiments of Sommer (43) and Lipman and McKinney (23) showed convincingly for the first time that plants demand copper for their normal development. Various investigations demonstrated that the growth of lower organisms (grey mould, yeast, algae, and bacteria) are greatly stimulated by slight quantities of copper (30, 6, 25, 34).

Copper probably plays a rôle in the oxidization processes, and it is not impossible that it also exercises an influence on certain vitamins. Schrenk (36) found an increase of oxidizing enzymes in kale sprayed with copper ammonium carbonate, whilst McHargue and Calfee (24) indicate that they found a relation between the occurrence of copper and vitamin A.

According to a statement of Scharrer and Schropp (35), Wieland (46) should have proved that copper has a catalytic reaction in the oxidizing processes, whilst Wertheimer (45) found that the oxidization of phenyleendiamin + α Naphtol to the pigment Indolphenol blue was caused by catalytic reaction of copper.

It is very probable that the function of copper in the plant and that in animals are analogical. The above investigation has shown that copper has an essential function in the formation and action of chlorophyll, as is probably the case with hemoglobine.

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THE LONGEVITY OF SMUT SPORES IN HERBARIUM SPECIMENS¹

GEORGE W. FISCHER²

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INTRODUCTION

It has long been known, in a general way, that the spores of some smut fungi possess considerable longevity when stored under conditions of moderate temperature and low relative humidity. This conception is based chiefly on fragmentary records of about 75 years.

In 1879 von Liebenberg, according to de Bary (1), indicated the viability of the spores of several smut fungi, including a demonstration of the longevity of $7\frac{1}{2}$ years for *Ustilago carbo*, (according to McAlpine (3) this record refers to *U. avenae* (Pers.) Jens.); $5\frac{1}{2}$ years for *U. kolaczekii* Kühn and *U. crameri* Körn.; $3\frac{1}{2}$ years for *U. rabenhorstiana* Kühn; $6\frac{1}{2}$ years for *Urocystis occulta* (Wallr.) Rab. and *Sphacelotheca sorghi* (Lk.) Clint.; and $8\frac{1}{2}$ years for *Tilletia tritici* (Bjerk.) Wint. de Bary (1) pointed out that since this record of *T. tritici* represented the oldest material von Liebenberg had at his command, and since the germination was so vigorous, it might be assumed that still older spores would have shown good germination. According to de Bary (1), Brefeld, as early as 1883 showed that the spores of *Sorosporium reilianum* (Kühn) McAlpine could retain their viability for 8 years.

McAlpine (3) has added several records of the longevity of smut spores: *Tolyposporium bursum* (Berk.) McAlpine 4 years; *Ustilago readeri* Sydow 2 years; *U. bromivora* (Tul.) F. d. W. at least $2\frac{1}{2}$ years; *U. cynodontis* Hem. at least 1 year; *Sorosporium consanguineum* Ell. and Ev. 3 years; and *Cintractia densa* McAlpine almost 5 years.

The foregoing longevity records have been supplemented in more recent years, by more or less scattered reports from various investigators, especially of the cereal smuts. Most of these reports have been incidental to other phases of research on smut fungi. Sobel (8) reports a longevity of 4 years for *Sphacelotheca cruenta*, but only 2 years for *S. sorghi*. Novak (5) reports almost unimpaired viability for *Tilletia levis* Kühn and *T. tritici* over a period of several years (exact number not given). Sobel (8) obtained germination of 7-year-old spores of *Tilletia tritici*, and Woolman and

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Humphrey (10) have shown that the spores of both species of *Tilletia* can retain their viability for 12 years or more.

Verwoerd (9) found that the spores of *Urocystis tritici* Körn. remain viable at least 5 years. Noble (4) augmented this record by obtaining vigorous germination of 10-year-old spores.

Spores of the oat-smut fungi likewise have been shown to possess considerable longevity. Sampson (7) reported only slight loss of viability for *Ustilago avenae* after 2 years; while Sobel (8) found that occasional spores 9½-years old would germinate. The records would indicate a greater longevity for *U. levis* (K. and S.) Magn. than for *U. avenae*. Thus, Sampson (7) found still viable the spores of a collection of *U. levis*, 5½ years old. Sobel (8) raised this record for *U. levis* to 13½ years.

According to Rump (6), *Ustilago hordei* (Pers.) K. and S. spores were still viable after 5 years, and Sobel (8) reported at least 7 years longevity for this species. *U. nuda* (Jens.) K. and S., on the other hand, seemed to be comparatively short-lived. Zeiner (11) reported germination of spores only 1 year old.

Benigni (2) was unable to obtain germination of the spores of *Ustilago zeae* (Beckm.) Unger older than 3 years.

Incidental to examining herbarium specimens of various smut fungi, the writer observed that a few collections that were several years old, contained a fair percentage of viable spores. Interest in the longevity of smut spores was thus aroused, and a survey of the literature revealed the fragmentary records presented above. It was then decided to make a concentrated study of the longevity of the spores of specimens of the Ustilaginales in the plant pathology herbarium of the State College of Washington, in an effort to supplement the above records with more exhaustive data.

MATERIALS AND METHODS

With 2 or 3 exceptions (representing relatively recent collections) the data obtained from the viability tests in the present investigation were taken from herbarium specimens. Presumably, such specimens are authentic as to identity and date of collection, and should serve as reliable material to establish age limits of spore viability. Using poured plates, (Petri dishes), the spores of the Ustilaginaceae were tested on potato-dextrose agar, while those of the Tilletiaceae were tested on plain agar. As a precaution against including foreign smut spores in any test, spore material was taken from unbroken sori with a sterile needle or loop. In order to avoid removing the covers and to minimize the danger of contamination with other fungi, the plates were inverted and the spores examined through the bottom. Of the available specimens 387 collections were selected for tests, and of these, 80 were found to contain viable spores.

RESULTS

The results are presented in tabular form. Table 1 includes a list of the species tested and the ages of the specimens. The ages of specimens containing viable spores are in bold-face type. The numerous duplications are not shown.

The data obtained from the 80 specimens showing germination are presented in tables 2 and 3. The source of each specimen also is shown.

Following are the species of smut fungi tested for spore viability. The figures represent the age of the various specimens; those in bold-face type represent the age of specimens containing viable spores. **USTILAGINACEAE:** *Cintractia angulata* Syd. 12; *C. caricis* (Pers.) Magn. 34, 12, 8, 5, 4, **4, 3**; *C. junci* (Schw.) Trel. 28, 26, 24, **3**; *C. luzulae* (Sacc.) Clint. 11, 3; *Melanopsichum austro-americanum* (Speg.) G. Beck 29; *Schizonella melanogramma* (DC.) Schröt. 25; *Sorosporium melandrii* Syd. **2**; *S. ellisii* Wint. 28; *S. everhartii* Ell. and Gall. 27; *S. reilianum* (Kühn) McAlpine 38, 30, 16, **2**; *S. saponariae* Rudolphi 16, 12; *S. syntherismae* (Peck) Farl. 33, 24, 9, 8, 7, **6, 5, 4, 3**; *Sphacelotheca andropogonis* (Opiz) Bubak 11, 6; *S. hydropiperis* (Schum.) de Bary 50, 34, 11, 10, 9, 5, 4; *Sphacelotheca ischmaei* (Fkl.) Clint. 35, 5; *S. occidentalis* Seym. 25; *S. sorghi* (Lk.) Clint. 38, 25, 14, **13, 9, 7**; *Thecaphora cuneata* (Schof.) Clint. 26; *T. deformans* Dur. and Mont. 20; *T. trailii* Cke. 30; *Tolyposporium bullatum* Schröt. 36, 3; *T. junci* (Schröt.) Woronin 16; *T. leptideum* Syd. 10; *Ustilago anomale* J. Kunze 42, 11, 10, 4, 3; *U. aristidae* Pk. 35, 34; *U. avenae* (Pers.) Jens. 26, 22, 17, 16, 14, **13, 12, 7, 4**; *U. betonicae* Berk. 25, 10; *U. bromivora* (Tul.) F. d. W. 43, 32, 30, 14, 12, **10, 9, 7, 4, 2**; *U. claytoniae* Shear 9; *U. crameri* Körn. 40, 37, 16, 9, **4**; *U. cynodontis* Hem. **5**; *U. echinata* Schröt. 13; *U. grandis* Fries. 12; *U. hordei* (Pers.) K. and S. 24, **23, 22, 17, 16, 14, 13, 11, 7, 5, 3, 2**; *U. hypodites* (Schl.) Fries 47, 34, 13, 9, 7; *U. levis* (K. and S.) Magnus 39, 32, 24, 23, 17, 9, **4**; *U. longissima* (Sow.) Tul. 13, 11; *U. lorentziana* Thüm. 31, 24, 23, 15, 9, **3**; *U. major* 13, 11; *U. marginalis* (DC.) Lév. 1; *U. neglecta* Niessl. 46, 26, 9, 7, **6, 5, 4, 3**; *U. oxalidis* E. and T. 28, 7, 6, 5, 4, 3; *U. perennans* Rostr. 37, 16, 14, 12; *U. pustulata* (DC.) Wint. 10; *U. nuda* (Jens.) K. and S. 37, 25, 17, 7, 4; *U. rabenhorstiana* Kühn 45, 23, 12, **8, 7, 5, 4, 3**; *U. residua* Clint. 6; *U. striaeformis* (West.) Niesl. 16, 11, 9, 5, 4, 3; *U. succisae* P. Magnus 9; *U. tritici* (Pers.) Rostr. 25, 20, 16, 11, 6, 4; *U. utriculosa* (Nees) Tul. 28, 14, 11, 10, 7, 6, 5, 4; *U. warmingii* Rostr. 3; *U. violaceae* (Pers.) Fkl. 16, 11, 10; *U. zae* (Beckm.) Unger 41, 27, 14, 9, **2**. **TILLETIACEAE:** *Entyloma calendulae* (Oud.) de Bary 11; *E. dahliae* Sydow **10**; *E. eryngii* (Cke.) de Bary **11**; *E. irregulare* Johans. 5; *E. lineatum* (Cke.) Davis 10; *E. physalidis* Wint. 10; *Doassansia alismatis* (Nees) Cornu 11; *D. sagittariae* (West.) Fisch. 11; *Tilletia anthoxanthi* Blytt 5, 4; *T. asperi-*

TABLE 1.—Summary of successful germination tests of smut spores—*Ustilaginaceae*

Species	Place of collection	Age of spores yrs.	Approximate percentage of germination on potato-dextrose agar after no. days indicated:				
			1 day	2 days	4 days	7 days	12 days
<i>Cintractia caricis</i>	Mehoopany, Pa.	4	0	0	0	0.1	—
“ “	Sweden, near Storlien	3	0	0	0.1	0.1	0.1
“ “	“ “	3	0	0	0.1	0.1	0.1
<i>Cintractia junci</i>	“ “	3	0	0	5.0	8.0	12.0
<i>Sorosporium melandrii</i>	Scotia, Pa.	2	0	1.0	10.0	10.0	—
“ “	Germany, Rheinprovinz:	2	0	45.0	*	*	—
“ “	Köln-Zollstock	7	0	0	0.1	0.1	—
<i>Sorosporium reilianum</i>	Pullman, Wash.	6	0	0	0	1.0	—
<i>Sorosporium syntherismae</i>	Chambersburg, Pa.	7	0	0	0	1.0	—
“ “	Lewistown, Pa.	6	0	10.0	30.0	*	—
“ “	Williamsport, Pa.	6	0	0	0.1	1.0	—
“ “	Sunbury, Pa.	5	0	0	8.0	*	*
“ “	Funkhannock, Pa.	5	0	1.0	40.0	50.0	—
“ “	Milford, Pa.	4	0	2.0	5.0	*	—
“ “	Sunbury, Pa.	3	0	1.0	20.0	*	—
“ “	Lock Haven, Pa.	13	10.0	40.0	*	*	—
<i>Sphacelotheca sorghi</i>	Daisy, Wash.	7	0	0	0.1	0.5	*
“ “	State College, Pa.	13	0	0	5.0	10.0	—
<i>Ustilago avenae</i>	Puyallup, Wash.	12	0	0	0	0.1	—
“ “	Germany, Rheinprovinz:	4	0	0	0.2	0.5	*
“ “	Ruwer bei Trier	4	0	0.1	0.5	*	*
“ “	Richardsville, Pa.	4	0	0.1	0.5	*	*
“ “	Milheim, Pa.	4	0.5	15.0	25.0	*	*
“ “	Centre Hall, Pa.	4	0.5	15.0	25.0	*	*

TABLE 1.—Summary of successful germination tests of smut spores—*Ustilaginaceae*—(Continued)

Species	Place of collection	Age of spores yrs.	Approximate percentage of germination on potato-dextrose agar after no. days indicated:				
			1 day	2 days	4 days	7 days	12 days
<i>Ustilago bromivora</i>	Waterville, Wash.	10	40.0	50.0	75.0	*	*
"	Walla Walla, Wash.	10	85.0	95.0	*	*	—
"	Munich, Bavaria	7	0	0	0.1	0.5	1.0
"	Pullman, Wash.	2	70.0	70.0	90.0	*	—
"	" "	2	60.0	60.0	90.0	*	—
<i>Ustilago crameri</i>	State College, Pa.	4	0	95.0	*	*	*
<i>Ustilago cynodontis</i>	Temple, Texas	5	0	0	0.2	5.0	—
"	" "	5	0.01	0.5	10.0	*	*
<i>Ustilago hordei</i>	Bozeman, Mont.	23	0	0	0	0	1.0
"	Puyallup, Wash.	17	0	18.0	50.0	*	*
"	Vancouver, Wash.	16	0	0.1	20.0	—	—
"	Monroe, Wash.	14	0	0	0.5	1.0	—
"	Germany, Rheinprovinz: Casel bei Trier	13	0.5	1.0	2.0	10.0	*
"	Pullman, Wash.	11	90.0	*	*	—	—
"	Wilcox, Pa.	7	45.0	75.0	85.0	*	—
"	Hyde, Pa.	7	80.0	95.0	*	*	—
"	St. Marys, Pa.	5	35.0	90.0	*	—	—
"	Ridgway, Pa.	5	45.0	60.0	*	*	—
"	Pullman, Wash.	3	80.0	95.0	*	—	—
"	" "	2	95.0	*	*	—	—
<i>Ustilago levis</i>	Kylestown, Pa.	4	0.5	1.0	1.0	10.0	*
"	Bellefonte, Pa.	4	10.0	40.0	50.0	*	—

TABLE 1.—Summary of successful germination tests of smut spores—*Ustilaginaceae*—(Continued)

Species	Place of collection	Age of spores yrs.	Approximate percentage of germination on potato-dextrose agar after no. days indicated:					
			1 day	2 days	4 days	7 days	12 days	
<i>Ustilago lorentziana</i>	Salmon, Idaho	3	50.0	*			—	*
<i>Ustilago mulfordiana</i>	Kettle Falls, Wash.	9	0	0	0	60.0	0.5	0.5
“	“	9	0	0	0	0.1		0.5
<i>Ustilago neglecta</i>	Beaver Falls, Pa.	7	0	0	0	0	0	30.0
“	Youngsville, Pa.	7	0	0	0	0	0	0.1
“	Orange, Pa.	7	0	0	0	0	0	—
“	State College, Pa.	6	0	0	0	0	0	0.2
“	North East, Pa.	6	0	0	60.0	75.0		*
“	Wayne Co., Pa.	5	0	0	40.0	80.0		*
“	Potter Co., Pa.	4	0	0	0	90.0		*
“	Burgettstown, Pa.	3	0	0	90.0	*		—
“	St. Lawrence, Pa.	3	0	0	0	0.1		—
“	Winsor, Conn.	8	0	0	0	1.0		—
<i>Ustilago rabenhorstiana</i>	Monaca, Pa.	7	0	0.1	1.0	40.0		*
“	Mehoopany, Pa.	5	0	0	5.0	1.0		*
“	Bowmansdale, Pa.	5	0	0	1.0	60.0		*
“	Jamestown, Pa.	4	0	0	0.1	*		*
“	State College, Pa.	3	0	0	80.0	—		—
<i>Ustilago zeae</i>	Pullman, Wash.	2	90.0	*	—			—

* Impossible to determine any increase in percentage of spore germination because of the maze of mycelium, or bud sporidia or both. On week-old plates contaminating fungi often hindered observations.

— Not examined further.

TABLE 2.—Summary of successful germination tests of smut spores—*Tilletiaceae*

Species	Place of collection	Age of spores yrs.	Approximate percentage of germination on plain agar after no. days indicated:							
			1 day	2 days	4 days	7 days	12 days	21 days	30 days	
<i>Entyloma dahliae</i>	Munich Botanic Gard.	10	0	0	0	1.0	8.0	10.0	—	
<i>Entyloma eryngii</i>	Germany: Cuxhaven, Hamburg	11	0	0	0	5.0	25.0	25.0	*	
<i>Tilletia levis</i>	Stockton, Kansas	25	0	0	0	0	5.0	20.0	*	
"	Guelf, Ont.	18	0	0	0	1.0	75.0	*	*	
"	Beaver, Utah	17	0	0	0	0	2.0	50.0	*	
"	Lewisburg, Pa.	6	0	0	0	5.0	60.0	*	*	
"	Fogelsville, Pa.	4	0	0	1.0	95.0	*	*	*	
<i>Tilletia ranwenhoffii</i>	Brandenburg, Germany	12	0	0	0	0	0	7.0	—	
<i>Tilletia separata</i>	Moscow, Russia	12	0	0	0	0	0	0	10.0	
<i>Tilletia trifici</i>	Coupeville, Wash.	18	0	0	0	5.0	60.0	*	—	
"	Thornton, Wash.	17	0	0	0	10.0	60.0	*	—	
"	Goldendale, Wash.	17	0	0	0	5.0	50.0	60.0	*	
"	Pleasant Prairie, Wash.	16	0	0	0	20.0	90.0	*	*	
"	Ridgefield, Wash.	16	0	0	0	1.0	80.0	*	*	
"	Lowden, Wash.	16	0	0	0	5.0	75.0	*	*	
"	Port Angeles, Wash.	16	0	0	0	1.0	30.0	60.0	*	
"	Germany: Trier, Rhein-provinz	13	0	0	0	0	40.0	*	*	

* The maze of mycelium and germinating secondary sporidia prevented the determination of any increase in percentage of germination.

— Not examined further.

folia E. and E. 16, 15, 10, 9; *T. holci* (West.) Rostr. 17, 16, 10, 9, 7, 6, 5; *T. levis* Kühn 44, 42, 25, 24, 23, 18, 17, 6, 4; *T. guyotiana* Har. 16, 10; *T. rauwenhoffii* F. d. W. 13, 12, 10, 9; *T. secalis* (Cda.) Kühn 12; *T. separata* J. Kunze 12; *T. tritici* (Bjerk.) Wint. 42, 18, 17, 16, 13, 12; *Tubercinia anemones* (Pers.) Liro 12; *T. cepulae* (Frost) Liro 12; *T. colchici* (Schlecht.) Liro 13; *T. ficariae* (Unger) Liro 12; *T. hellbori-viridis* (DC.) Liro 11; *T. hepaticae-trilobae* (DC.) Liro 12.

DISCUSSION

An analysis of the data presented in the preceding paragraph and in tables 1 and 2 reveals a surprising spore longevity for some smut fungi. Of the 23 species showing viable spores from herbarium specimens, *Tilletia levis* possessed the greatest longevity. A collection made at Stockton, Kansas, 25 years ago, showed 20 per cent germination after 21 days. Several collections of *T. levis* and *T. tritici*, 16 to 18 years old, showed 50-75 per cent germination after 12 or more days.

While, in general, the greatest longevity seems to be possessed by species in the Tilletiaceae, certain of the Ustilaginaceae are capable of retaining their viability over remarkably long periods of time. The greatest distinction goes to *Ustilago hordei*, of which 16- and 17-year-old specimens showed 20 per cent and 50 per cent germination, respectively, after 4 days, while a 23-year-old specimen showed 1 per cent germination after 12 days.

The results of the present investigation establish a number of new records for spore longevity in the smut fungi. The greatest reported heretofore for *Tilletia levis* and *T. tritici* was 12 years (10). The data presented in this paper extend this to 25 years and 18 years for *T. levis* and *T. tritici*, respectively. The greatest longevity established heretofore for *Ustilago hordei* was 7 years (8); the present data show that this species can retain slight viability for 23 years. Sobel (8) established the longevity of *U. avenae* at 9½ years; in the present investigation a 13-year-old specimen showed 10 per cent germination after 7 days. The same author (8) reported a retention of viability of only 2 years for *Sphacelotheca sorghi*. The writer obtained 40 per cent germination from a specimen 13 years old, and 0.5 per cent germination from one 7 years old.

On the other hand, for a few species, tests by the writer failed to equal the longevity records established by other investigators. Thus, both Sampson (7) and Sobel (8) have established a greater longevity for *Ustilago levis* than for *U. avenae*. The former obtained germination from collections 5½ years old, and the latter from a collection 13½ years old. The author was unable to obtain germination from collections more than 4 years old.

Spore longevity tests remind one again of the danger of introducing plant pathogens through herbarium specimens, when these are improperly

cared for after being received. Eleven of the 80 herbarium specimens showing viability came from foreign countries; of these 11 specimens, 7 are now 10 years or more old. Disinfection of herbarium specimens with carbon tetrachloride or carbon disulphide, and subsequent storage with naphthalene crystals, apparently do not destroy the viability of smut spores.

Age certainly is not the only factor controlling the viability of the spores of the smut fungi. A collection of *Sphacelotheca sorghi* 13 years old showed 40 per cent germination after only 2 days, while another collection only 7 years old showed a maximum of only .5 per cent germination after 7 days. A collection of *Ustilago avenae* 13 years old gave 10 per cent spore germination after 7 days; another only 4 years old showed only .5 per cent after the same length of time. Two collections of *Ustilago bromivora*, 10 years of age, showed 75 per cent and 95 per cent germination, respectively, after only 4 days, while collections 9 and 4 years old showed no germination, even after 12 days on potato-dextrose agar. Also, of 5 specimens of *Tilletia tritici* 17 years old, 2 showed 50 to 60 per cent germination after 12 days, while the other 3 specimens showed no germination. Numerous other examples from the data could be cited to illustrate the point. Sampson (7) is of the opinion that for a given species (at least in the oats smuts) the state of maturity at the time of collection determines to a large extent the longevity of a given sample. *Within a species*, then, fully mature spores could be expected to retain their viability a much longer time than partially mature spores. The factors that cause the marked differences in spore longevity among the various species of smut fungi are not known and the present investigations throw no light upon this problem.

SUMMARY

Three hundred and eighty-seven herbarium specimens of 77 species of smut fungi were tested for longevity of their spores.

Of these, 80 specimens, representing 24 species, were found to contain viable spores. Eleven of these specimens were from foreign countries.

Some species were found to retain their viability for a long time. Noteworthy among these are: *Tilletia levis*, 25 years; *T. tritici*, 18 years; *Ustilago hordei*, 23 years; *U. avenae*, 13 years; *Sphacelotheca sorghi*, 13 years; and *U. bromivora*, 10 years. These records are much higher than those established by previous investigators.

Considerable differences in viability were noticed in various collections of the same age and of the same species. Such differences are thought to be correlated with the degree of maturity of the spores at the time of collection.

In several instances collections 12 to 18 years old would show decidedly greater percentage of germination than much younger collections

of the same species. This would seem to lend support to Sampson's (7) opinion that *within a species* the state of maturity at the time of collection determines, in large measure, the longevity of the spores.

In general, the species belonging to the Tilletiaceae are possessed of greater longevity than those belonging to the Ustilaginaceae.

DEPARTMENT OF PLANT PATHOLOGY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON.

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ANGUILLULINA PRATENSIS IN RELATION TO ROOT INJURY OF APPLE AND OTHER FRUIT TREES

P. A. ARK AND H. EARL THOMAS

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In the course of a study of dieback and rosette (little leaf (1)) of the apple, the fine feeding roots were found to be distinctly injured. The search for a specific cause revealed the presence (within the roots), in considerable number a nematode that was identified by Steiner as the meadow nematode, *Anguillulina pratensis* (De Man, 1880) Goffart, 1929, and reported by him as a new parasite for apple roots (6)¹. More recently this nematode has been found in the roots of plum, peach, pear, and grapes, and in these cases also in association with the little leaf disease. The roots of fig trees, however, seem to be particularly susceptible to direct injury by this parasite (2, 8), although this fruit tree is seldom found affected by little leaf. Moreover, among the many hosts previously known for *A. pratensis* (5), many species are not known to be affected by little leaf or dieback. Nevertheless, the obvious direct injury to the roots and the possible secondary rôle of the nematodes in the production of the above diseases seem to merit further study. A different species of nematode, *Tylenchulus semipenetrans* Cobb 1913, was found some years ago (7) in the roots of citrus trees affected by mottle leaf which now seems to be similar in nature to the little leaf of deciduous fruit trees.

Early symptoms appear as necrotic spots on the fine white rootlets, ranging from microscopic size to streaks several millimeters in length and from dark amber to black (Fig. 1). These symptoms are very similar to those produced on pineapple (3) by the same (4, 5) or a very similar nematode. Heavily infested roots usually are stunted and sometimes thickened and distorted. Among infected roots are commonly found tufts, such as that shown in figure 1-D, that resulted from the repeated development of branches that are in turn killed back. It is not clear, however, that all of this type of symptom is to be charged to the activity of the nematodes. The larger roots of the apple and stone fruit trees that have been examined, unlike those of figure 1, exhibit no symptoms.

The nematodes invade only the cortex and align themselves more or less parallel with the axis of the rootlet. Eggs and larvae in all stages of development may be found together (Fig. 2).

¹ Steiner, G., and E. M. Buhner. Observations on nematode diseases of plants. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. 19 (3): 24-25. 1935. [Mimeographed.]

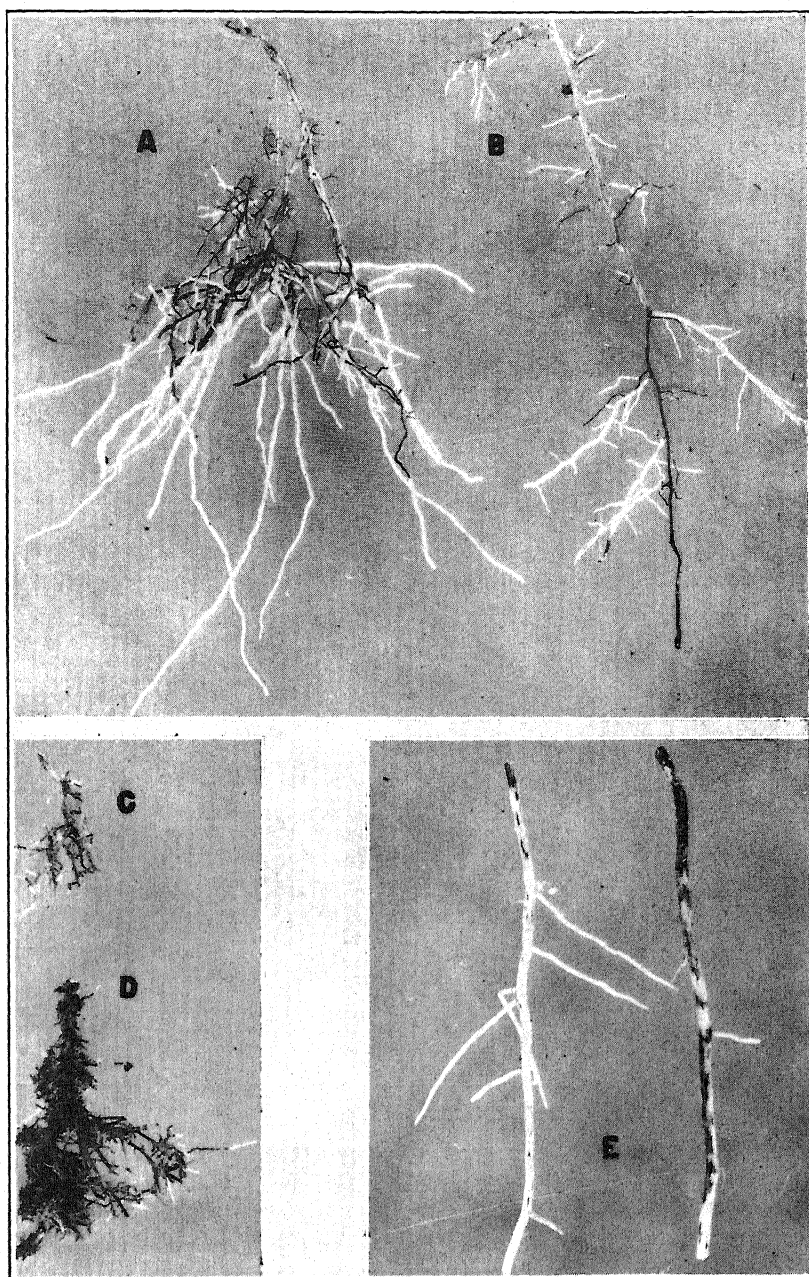


FIG. 1. A-B. Apple roots from a healthy orchard (Elphic's, Sonoma County). Note long, slender, widely spread healthy rootlets. C-E. Apple roots of trees with dieback symptoms (McKenzie's orchard, Sonoma County). In C and D note stunted condition and new rootlets of dwarfed type. In E note numerous blackish and dark brown lesions. These numerous black streaks may form large and sunken lesions. \times approximately 1.

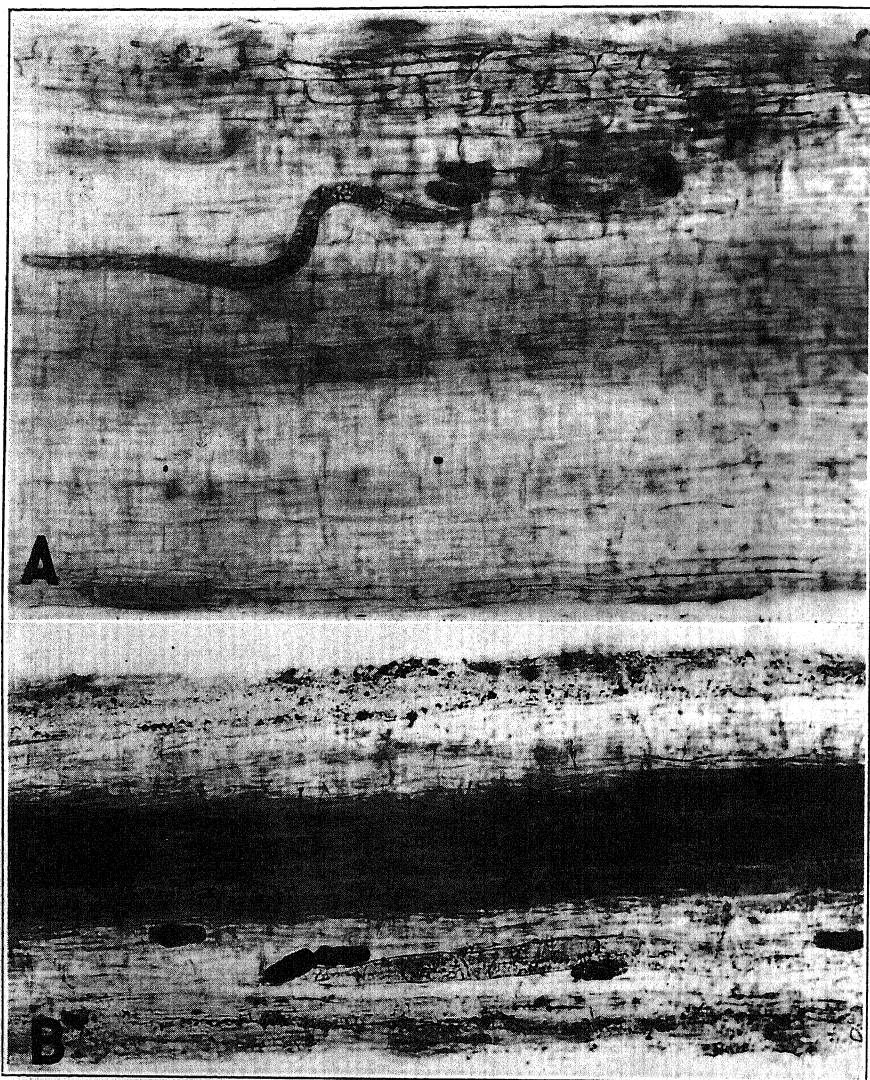


FIG. 2. Apple rootlets. A. Intracellular nematodes and their eggs prepared by modified Godfrey's method. B. Nematode and eggs in another root. \times approximately 125.

In fixed material of apple rootlets stained with Flemming's triple stain, bacteria were found abundant, not only behind and in the immediate vicinity of the nematode, as has been noted by other workers (3) (8), but also to 10 or 15 cells distant from the nematode or its path. In the latter case the cells, by which the bacteria were surrounded, bore no evidence of disintegration. A few cells near the nematode were collapsed and filled with bacteria (Figs. 3, B and 4).

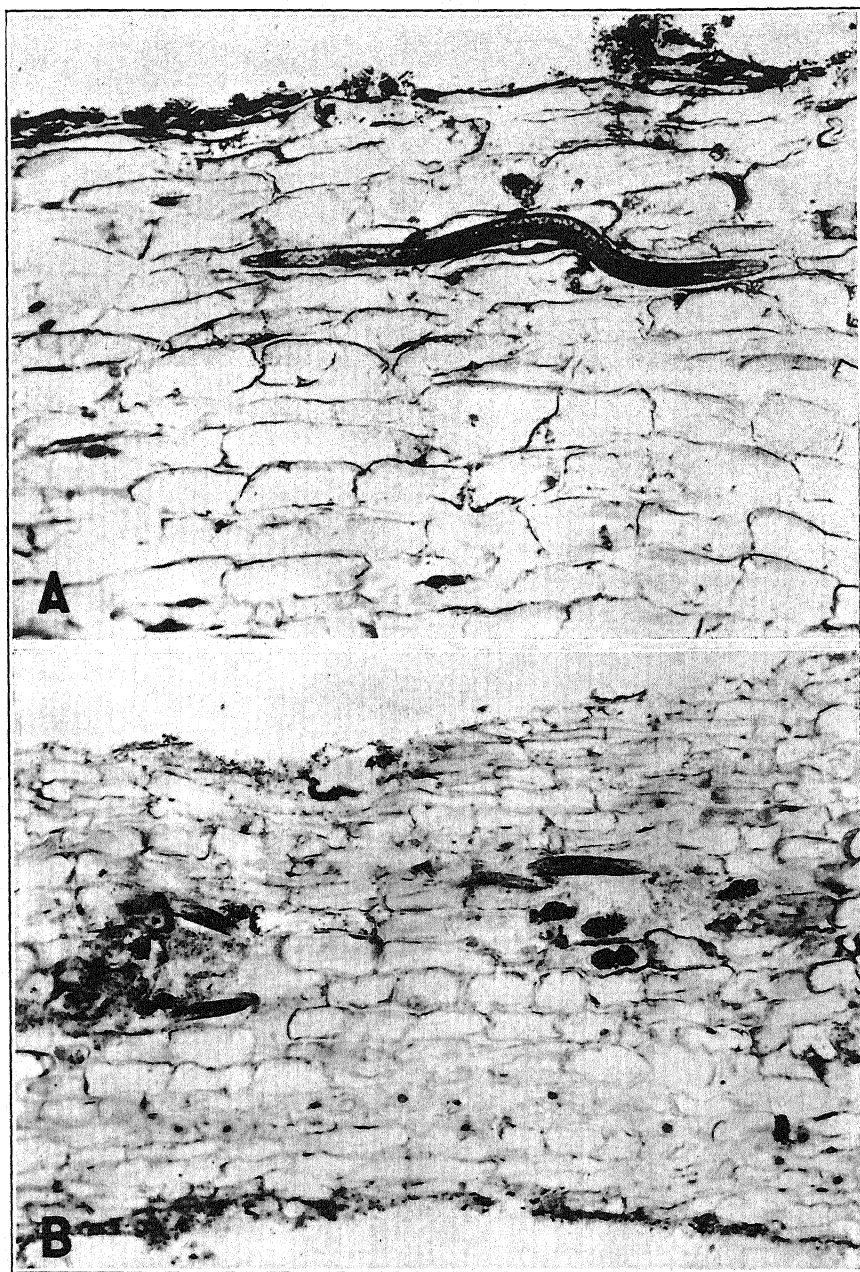


FIG. 3. A. Longitudinal section of a rootlet infested with nematodes. Section 8μ thick and stained with the Gentian violet stain. Note the breaking of cell walls as nematode paved its way. \times approximately 125. B. Longitudinal section through the cortex of a rootlet showing nests of nematodes and their eggs. Sections 8μ thick and triple-stained. \times approximately 125.



FIG. 4. A. Small portion of a section shown in figure 3, A. Abundance of bacteria in cells occupied by a nematode and also in many cells away from it. Sections 8μ thick and triple-stained. B. Bacteria in cells infested with a nematode. Stoughton's method. Sections 8μ thick. $\times 1000$.

Two different species of bacteria were consistently associated with this nematode in infested roots. One species is identified as *Pseudomonas fluorescens* (Flügge) Migula, while the taxonomic position of the second has not been determined. The characters for the second organism are as follows: A short rod, motile by one polar flagellum, measuring $1\ \mu$ – $1.9\ \mu \times 0.6\ \mu$ – $0.9\ \mu$, nonsporiferous; Gram-negative, grows well in most of the common bacteriological media; it grows as grayish white film on beef-agar slants, the growth being more abundant on potato-dextrose (2 per cent)-peptone agar slants; it produces acid and gas in dextrose, sucrose, maltose, galactose, mannite, and arabinose; grows well in Fermi's solution; no growth in Cohn's and Uschinsky's solutions; gelatin liquefied; indol produced and nitrates reduced; no diastatic action on starch. It grows at 10°C ., but not at 5°C ., optimum for growth being 30°C . The upper thermal death point is 46°C .

The same organisms were obtained from apple roots experimentally infested with nematodes in the greenhouse. Inoculum was prepared by taking roots heavily infected with nematodes, washing and disinfecting them in a 1–1000 solution of HgCl_2 for 3 minutes, with subsequent washings in sterile distilled water, and teasing the tissues to free the nematode larvae into distilled water. This then was introduced into a sterilized soil with apple seedlings. It is impossible to conclude whether these bacteria are merely carried mechanically or associated in some more specific relation with the nematodes. Fifty apple seedlings grown in sterilized soil to which these species of bacteria were added separately or in combination in the absence of nematodes did not become diseased—their roots and tops remained normal.

In another experiment ten seedling apple trees were planted in each of the 3 following soils: soil heavily infested with *Anguillulina pratensis*, steam sterilized soil with nematodes, and the same kind of soil without the addition of nematodes. Plants in nonsterilized soil and sterilized soil plus nematodes, although lacking symptoms on leaves or stems, were decidedly less thrifty and made less growth than the plants in the sterilized soil without nematodes. All plants were fertilized with calcium nitrate.

The degree of injury caused by the nematode alone in the orchard, or even in the greenhouse, cannot be determined with certainty until methods are devised for freeing the nematodes from associated bacteria and other organisms. The conclusion seems inescapable, however, that a considerable amount of direct injury must follow the invasion of the feeding roots of fruit trees by large numbers of these nematodes.

A matter of possibly greater interest in relation to the particular orchards under consideration is the opening of avenues into the roots for the entrance of microorganisms, or for the absorption of preformed toxic materials from the organisms around the roots. That such toxic materials are present in the root zone has been demonstrated by the wilting of de-

tached apple shoots placed either in the filtered leachate of soil from around affected roots or in the filtrate from pure cultures of organisms isolated from the root zone of affected apple, apricot, and peach trees.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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POWDERY MILDEW (*ERYSIPHE POLYGONI*) ON GARDEN SNAP BEANS

W. D. MOORE^{1,2}

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INTRODUCTION

Powdery mildew (*Erysiphe polygoni* DC.) has been known for many years to attack both garden and field beans, but comparatively few workers have attached any particular importance to it. That the disease is widely distributed, however, is indicated in numerous references in the literature since 1889.

During the past 10 years, powdery mildew has become increasingly important throughout the southeastern part of the United States, particularly on the fall crop of beans, which is harvested during September, October, and November. In many cases the disease has caused a dwarfing of the plants, partial defoliation, and pod spotting, which has resulted in heavy losses to the growers. Since little or no systematic study had previously been devoted to the development and control of this malady, a series of field experiments to this end was started in 1931 and continued through 1935. These experiments were conducted near Charleston, South Carolina, at the substation of the State Agricultural Experiment Station, in a region largely devoted to the growing of vegetable crops for the market. It is believed that the data published here are applicable to the control of the disease in the territory included in the South Atlantic Seaboard.

HISTORICAL

The first announcement of damage from powdery mildew on beans was from Bermuda in 1889, by Galloway.³ Whetzel⁴ reported it again from the same place in 1922, stating that it was the most important disease on beans at that time. Sherbakoff⁵ noted a general infection in Florida in 1917, and also described briefly the pod spot that recently has become of considerable economic importance along the Atlantic Coast from Virginia southward.

¹ Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture.

² Acknowledgment is made to Dr. L. L. Harter, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, for valuable suggestions during the course of this work and for criticisms of the manuscript.

³ Galloway, B. T. Powdery mildew of the bean. Jour. Mycol. [U. S.] 5: 214. 1889.

⁴ Whetzel, H. H. Report of the Pathologist. Bermuda Bd. and Dept. Agr. Repts. 1921: 41. 1922.

⁵ Sherbakoff, C. D. Some important diseases of truck crops in Florida. Florida Agr. Expt. Sta. Bull. 139: 217-218. 1917.

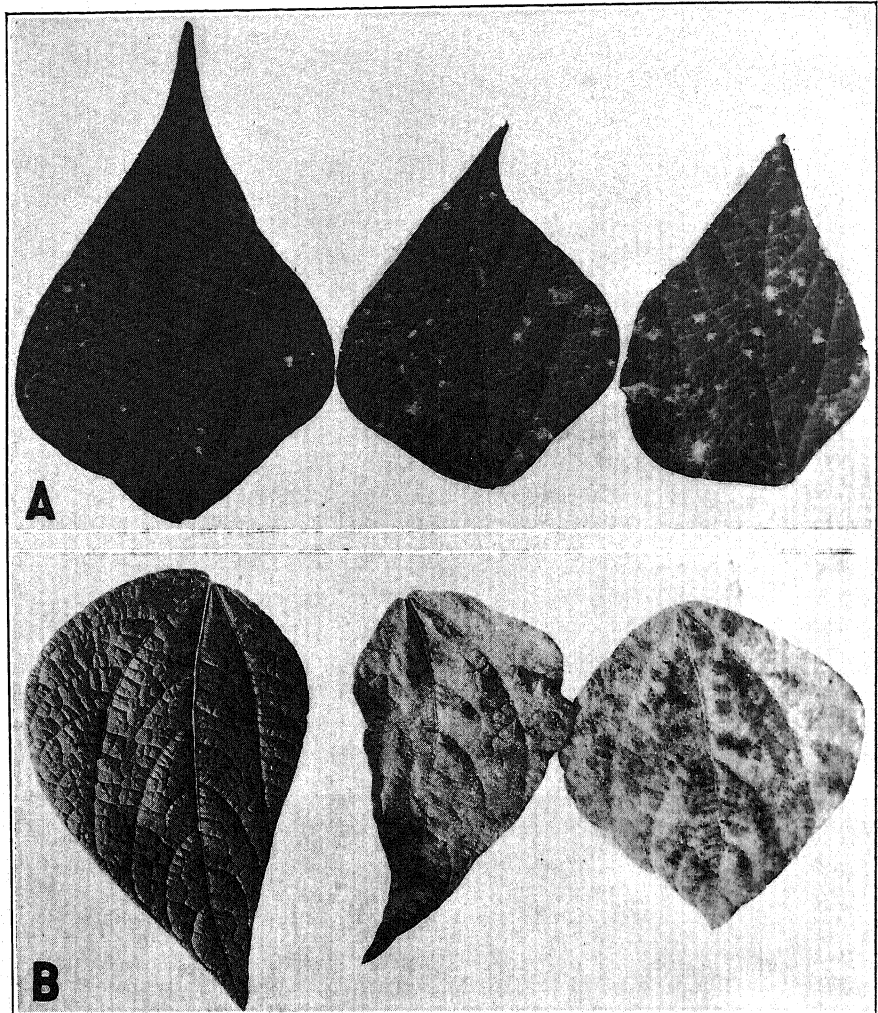


FIG. 1. A. Mature bean leaves showing initial stages of powdery mildew infection. Leaf on left shows spots about 1 day after first being visible. Those on right show spots 3 days old. B. Bean leaves showing advanced stage of infection. Note complete fungous covering of 2 diseased leaves on right as compared with healthy leaf on left.

Linford⁶ reported powdery mildew causing a dwarfing of the young pods in Utah in 1927. Cook⁷ described the powdery mildew pod spots as the

⁶ Linford, M. B. Powdery mildew damages beans. U. S. Dept. Agr. Bur. Plant Indust. Plant Disease Rept. 11: 137-138. 1927. [Mimeographed].

⁷ Cook, H. T. Powdery mildew disease of snap beans. Virginia Truck Expt. Sta. Bull. 74. 1931; The control of powdery mildew of snap beans. Peninsular Hort. Soc. [Del.] Trans. 46: (Delaware State Bd. Agr. Bull. V. 22 (5)): 25-27. 1932.

cause of heavy losses in Virginia in 1931. He described the disease, as it occurs in that section, in considerable detail and reported data on experimental control with both sulphur sprays and sulphur dusts. Numerous plant disease reports^s indicate that powdery mildew has become of considerable importance over a large section of the bean-growing territory since 1920, causing appreciable losses in many instances.

DESCRIPTION

The disease first appears on the upper surface of the older leaves as very small, white, circular, powdery spots (Fig. 1, A). These enlarge rapidly, coalesce to include larger areas, and finally cover the entire leaf (Fig. 1, B). In some instances infection may originate at points along the midrib or veins, the spots being circular, at first, then becoming elongate. The lower surface of the leaf may become infected at the same time as the upper, although this is not always the case. If infection occurs before the plants are mature, the leaves may become dwarfed, turn yellow, and finally fall off.

Fungus development on the stems and petioles is much the same in appearance as on the midribs and veins, although it usually follows foliage infection. The fungus grows rapidly over the surface of the leaves and, in advanced stages, the foliage appears to be covered with a talcum-like powder.

Powdery mildew may attack the immature pods just as it does the stems, causing serious dwarfing and premature death (Fig. 2, A). In such cases the fungus appears as a powdery coating over nearly all of the pod. It does not produce a distinct spot. This type of infection may be found in almost any generally infected field near the close of the harvest season but causes only slight losses, as a general rule. During certain seasons, and on the maturing pods, a more severe infection occurs, which begins as very small moist-looking circular spots. The spots enlarge rapidly, coalesce, and cause large irregular areas that may eventually cover the whole pod (Fig. 2, B). The early stages in the development of these spots show the powdery-appearing fungus growth, which soon becomes a light brown and finally a reddish brown. In severe cases the large spots may be slightly sunken.

EXPERIMENTAL MATERIALS AND METHODS

Experimental plots, each 100 feet long and 4 rows wide, were used in this study. All treatments were replicated 3 or more times, the plots in all cases being systematically distributed over the experimental area. Data

^s Tims, E. C. Powdery mildew (*Erysiphe polygoni*). U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Repr. 18: 94. 1934. [Mimeographed]. Barrus, M. F., O. C. Boyd and J. I. Wood. *Ibid.*, Sup. 81: 111. 1931. Miller, P. R., N. E. Stevens and J. I. Wood. *Ibid.*, 84: 32. 1933. Wood, J. I., N. E. Stevens and P. R. Miller. *Ibid.*, 85: 46. 1933.

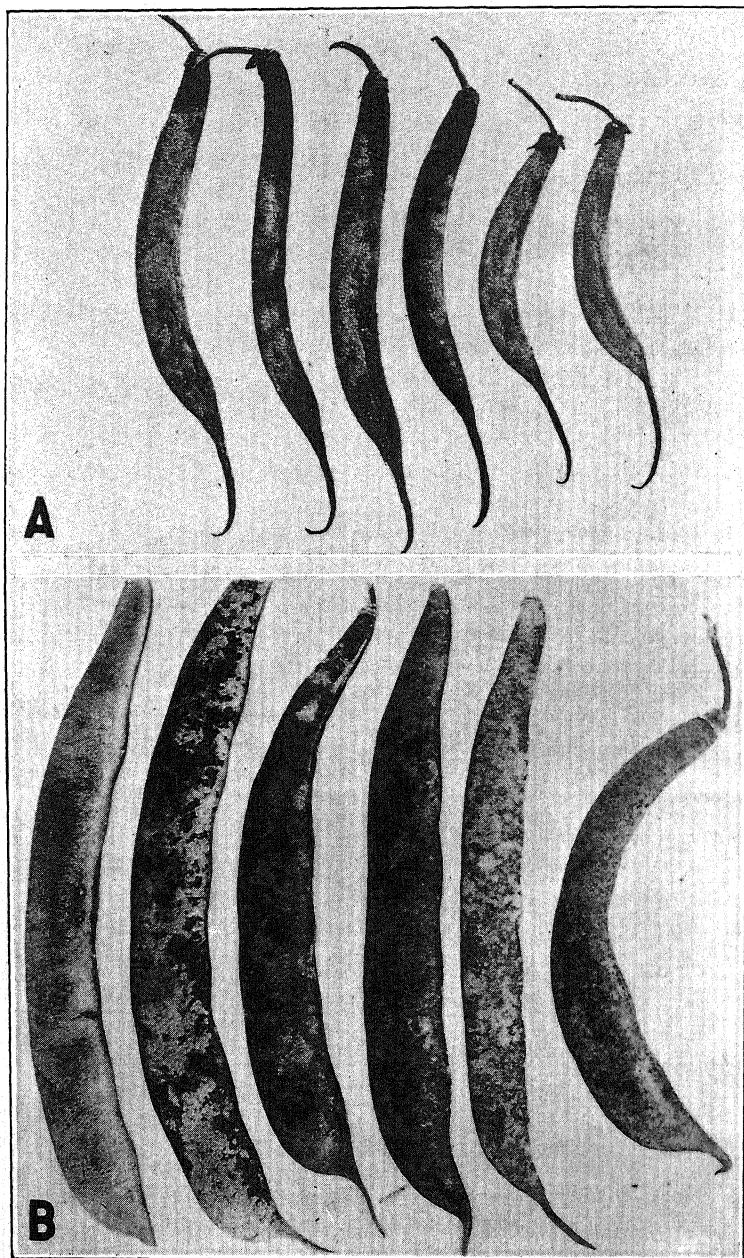


FIG. 2. A. Young bean pods showing heavy infection of powdery mildew. Note that the fungus had killed the immature pods. B. Mature bean pods showing various stages of spotting caused by powdery mildew.

on both foliage and pod infections were collected only from the 2 center rows of each plot. Dust materials, consisting of various mixtures of sulphur and lime, sulphur and colloidal clay, different commercial sulphur dusts, and copper-lime dust, were applied with rotary hand dusters at the rate of 25 pounds per acre. All dust applications were made in late afternoon or early morning, when there was sufficient moisture on the foliage to insure a thorough sticking of the different materials. The spray materials, consisting of lime-sulphur spray, Bordeaux mixture, and two concentrations of flotation sulphur, were applied with hand-spray machines at the rate of approximately 100 gallons per acre. These applications were made when there was no dew on the foliage. The first application of both sprays and dusts was made when the initial stage of mildew infections appeared on the leaves and subsequent applications followed at approximately 10-day intervals, depending upon rainfall. In all cases the disease appeared so late in the growing season that not more than 2 applications of the most

TABLE 1.—Combined data on powdery mildew foliage infection on beans when various dusts were used as controls in 1931 to 1935

Fungicide	Plants examined	Plants infected	Plants infected
Data for years 1931-1935			
Nontreated check	No. 5490	No. 4797	Per cent 87.3
Sulphur dust 100 per cent	2382	54	2.2
Sulphur-lime dust 75-25	2337	87	3.7
Sulphur-lime dust 50-50	2250	139	6.1
Sulphur-lime dust 25-75	2328	837	35.9
Data for years 1931-1934			
Nontreated check	4690	3997	85.2
Copper-lime dust 25-75	1816	777	42.7
Data for 1933-1934			
Nontreated check	1418	1241	87.5
Sulphur-clay 75-25	389	6	1.5
Sulphur-clay 50-50	343	23	6.7
Data for year 1932			
Nontreated check	800	800	100.0
Manganar Rose dust 85 per cent sulphur	400	41	10.2
Koppers sulphur dust	400	18	4.5
Kolodust	165	5	3.0
Data for year 1933			
Nontreated check	1391	1391	100.0
Koppers sulphur } 25-75	329	8	2.4
Electric sulphur }			
Koppers sulphur } 50-50	316	0	0.0
Electric sulphur }			

effective materials were necessary to protect the plants throughout the harvest period.

Of the several fungicides tested in this work, only the sulphur-lime mixtures were used throughout the 5-year period. Some materials were too expensive to be of economic value to the growers, while others proved to be inefficient or of inadequate physical condition for a thorough covering of the plants. As these weaknesses appeared, the materials were discarded in favor of the more promising ones.

Dust. In table 1, it seems that essentially all dusts used, containing 75 per cent or more of sulphur, gave a high degree of control for powdery mildew. The Koppers sulphur-electric sulphur combination of 50 per cent of each was superior to any of the materials used, but the cost of this material was too great when its efficiency was compared with that of the 75-25 sulphur-lime combination. The sulphur-clay mixtures, while giving a high degree of control, were objectionable because of a tendency of the dust to clog the dust guns. Pure sulphur was difficult to use for the same reason. Of the several dust materials used, the sulphur-lime 75-25 combination proved to be the most desirable control for powdery mildew when the fungicidal action, ease of handling, and economy of the material are taken into consideration.

TABLE 2.—*Combined data on powdery mildew foliage infection of beans where different sprays were used as controls for the years 1931 to 1935*

Fungicide	Plants examined	Plants infected	Plants infected
	<i>No.</i>	<i>No.</i>	<i>Per cent</i>
Nontreated check	2218	2041	92.0
Bordeaux mixture 4-4-50	1408	247	17.5
Lime-sulphur spray	1381	328	23.8
Flotation sulphur spray 1-160	1332	80	6.0
Flotation sulphur spray 1-320	975	131	13.4

Sprays. Of the four spray materials tested, the Flotation Sulphur 1-160 proved to be the most efficient (Table 2). Regular lime-sulphur (dry lime-sulphur 3-400) proved highly efficient in seasons of little or no rain, but did not give a good control during periods of intermittent rains. In no case did Bordeaux mixture give a desirable control for this disease.

DISEASE CONTROL

Even though there is considerable damage from powdery-mildew infection through the destruction of chlorophyll in bean leaves, together with

TABLE 3.—*Data showing number of bean pods examined, number and percentage of pods infected by powdery mildew where various sprays and dusts were used as controls for the years 1931 and 1932*

Fungicide	Pods examined	Pods infected	Pods infected
	<i>No.</i>	<i>No.</i>	<i>Per cent</i>
Nontreated check	2795	1498	53.6
Sulphur dust 100 per cent	877	0	0.0
Sulphur-lime 75-25	1002	13	1.2
Sulphur-lime 50-50	1003	15	1.4
Sulphur-lime 25-75	1003	18	1.7
Manganar rose dust 85 per cent sulphur	363	8	2.2
Copper-lime dust 25-75	1114	456	40.9
Koppers sulphur dust 100 per cent	402	0	0.0
Lime-sulphur spray	377	9	2.3
Bordeaux mixture 4-4-50	346	59	17.0
Flotation sulphur spray 1-160	319	5	1.5
Sulphur-aluminium stearate dust 95 per cent sulphur	650	0	0.0

some defoliation, the major economic losses are from pod spots. The principal aim, therefore, in controlling leaf, petiole, and stem infection is to protect the developing pods from infection. Since pod infection occurs considerably later in the season than does foliage infection and, since pod infection probably comes largely from the foliage, stems, and petioles, it is reasonable to expect pod-spot control to parallel very closely that of foliage infection. This is clearly seen from the data in table 3. Due to the fact that pod spot developed on the experimental plots in only 2 of the 5 years this work was done, the data are not so extensive as in the case of foliage infection. It is apparent, however, from the information collected that 100 per cent sulphur as a dust, 75-25 sulphur-lime dust, 100 per cent Koppers Sulphur dust, lime-sulphur spray or flotation sulphur spray 1-160 will give adequate control.

RELATION OF WEATHER TO DISEASE INCIDENCE

Powdery mildew occurs every year on beans in the Southeastern United States during the fall months, but rarely does it appear on the spring crop. The reason for this, though probably a matter of speculation, is, nevertheless, of some interest and importance. The number of spores is somewhat limited during the spring months as compared with the fall months, because of the earliness of the bean crop, the harvest period ranging from late April through May. Few of the hosts of the causal organism are developed at this time, consequently there is not a general distribution of spores. During

the fall months, however, there are numerous plant species, such as those from the genera *Brassica*, *Trifolium*, *Vigna*, *Pisum*, *Medicago*, and others, on which the same species of mildew develop early in September, thus furnishing ample spore material for a general field infection. With this natural condition prevalent, the factors limiting bean infection in the fall would logically appear to be either humidity or air temperature or both.

TABLE 4.—*Showing dates of mildew infection on beans for the years 1931-1935, date of rainfall, and elapsed time until infection occurred*

Rainfall	Rainfall	Mildew infection	Days between rainfall and infection	Mean temperature 10 days before infection	Mean temperature for month of May
<i>Date</i>	<i>Inches</i>	<i>Date</i>		<i>°F.</i>	<i>°F.</i>
10/ 9/31	0.18	10/13	4	73.8	71.4
10/ 5/32	0.53	10/ 8	3	69.6	72.6
10/ 9/33	0.13	10/16	7	67.8	77.9
10/ 1/34	1.77	10/ 4	3	76.0	71.0
9/19/35	0.20	9/24	5	76.0	73.6

In table 4 are shown the first date of observed mildew infection for each year from 1931 to 1935, inclusive, together with date of first rainfall and amount of rainfall prior to infection and the mean temperature for the 10-day periods immediately preceding infection. For comparison the mean monthly temperatures for May are shown for each of the above years. Here it appears that a correlation exists between humidity and disease infection during the fall bean season. Temperature conditions at all times seem to be within the range favorable for fungus development. With an abundance of spore material present on both cultivated and wild host plants, the spread to and the development on the bean plants seems dependent only upon a requisite amount of rainy weather. However, the data presented are too limited to warrant a definite conclusion on this point and are included to show only an apparent relationship.

VARIETAL SUSCEPTIBILITY

Although all of the known commercial varieties of snap beans are susceptible to powdery mildew, there is a wide difference in susceptibility. In table 5 are listed, according to severity, 33 of the better-known varieties on which observations of natural infection were made during the period of this investigation. Here it is seen that Asgrow Valentine and Sure Crop Wax showed light infections of powdery mildew, while Giant Stringless

TABLE 5.—*Showing the relative susceptibility of various dwarf varieties of beans to infection by powdery mildew*

Variety	Class	Kind of pod
Light infection ^a		
Asgrow Valentine	Garden	Green
Dwarf Horticultural	"	"
French Horticultural	"	"
Great Northern	Field	"
Grenell Rustproof	Garden	"
Hodson Wax	"	Wax
Keeney Rustless Wax	"	"
Prolific Black Wax	"	"
Round Pod Kidney Wax	"	"
Stringless Green Refugee	"	Green
Stringless Kidney Wax	"	Wax
Sure Crop Wax	"	"
Moderate infection		
Black Valentine	Garden	Green
Burpee Stringless Green Pod	"	"
Currie Rustproof Wax	"	Wax
Davis White Wax	"	"
Fordhook Favorite Bush	"	Green
Full Measure	"	"
Giant Stringless Green Pod	"	"
Improved Golden Wax	"	Wax
Konserva	"	Green
Longfellow	"	"
Pencil Pod Black Wax	"	Wax
Red Valentine	"	Green
Unrivalled Wax	"	Wax
Wardwell Kidney Wax	"	"
Weber Wax	"	"
Severe infection		
Bountiful	Garden	Green
Extra Early Refugee	"	"
Low Champion Bush	"	"
Refugee 1000-1	"	"
Refugee Wax	"	Wax
Tennessee Green Pod	"	Green
White-seeded Refugee	"	Wax

^a Infections from a trace to 25 per cent are listed as "light"; from 25 to 75 per cent as "moderate"; from 75 to 100 per cent as "severe."

Green Pod, Black Valentine, and Konserva were moderately infected. Bountiful was the only important southern commercial variety that was severely infected. It should be noted, however, that either a light or a moderate infection may be of considerable economic importance, since infected bean pods were found on plants that showed only a limited amount of foliage infection, as well as on those severely infected. While this grouping of varieties is arbitrary and subject to some change from year to year, it does suggest a degree of varietal resistance that may be of importance in a program of plant breeding.

DISCUSSION OF RESULTS

Powdery mildew attacks the fall crop of beans throughout the Southeast. It seldom is observed on the spring crop, although both rainfall and mean temperatures for May and October (Charleston, S. C., readings) are so nearly the same as to indicate that factors other than weather may cause the difference in mildew infection. The wide distribution during the fall of natural host plants of *Erysiphe polygoni*, which produce an abundance of spores during the later stages of bean growth, seems to account for this seasonal occurrence. That only a limited amount of rainfall is necessary for infection is indicated from the weather data collected over the 5-year period covered by this work. It is not unlikely that the length of the period of high humidity during a rain or following it might be in part at least the factor controlling infection.

Since powdery mildew may be expected to appear in bean fields throughout the Southeast each fall, and since infection consistently follows rains during late September and early October, the time for inaugurating control measures can be fairly well ascertained. The data collected on control of powdery mildew indicate that either sulphur sprays or sulphur dusts may be used, but that the sulphur-lime dust (75 per cent sulphur, 25 per cent lime) is more effective, easier to handle and more economical than the sprays. Good control may be had by making the first application when the first signs of the disease appear on the foliage, and repeating at 10-day to 2-week intervals thereafter. Usually, from two to three applications should be sufficient.

SUMMARY

Powdery mildew is a widely distributed disease of snap beans, and, in recent years, has become of considerable economic importance throughout the Southeast.

The disease is rarely found on the spring crop, but appears each year on the fall crop.

Fungus development is closely associated with moisture conditions, usually following upon light rains in late September and early October.

Good control of the disease may be obtained by application of either sulphur dusts or sulphur sprays. The sulphur-lime 75-25 dust proved to be the most economical and efficient material tested.

A VIRUS DISEASE OF PRUNE

H. EARL THOMAS AND E. M. HILDEBRAND

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During the late summer of 1930, attention was called to a disease of prunes in Niagara County, New York, which was accompanied by certain symptoms characteristic of virus diseases. Thus far these symptoms have been seen occurring naturally only in Niagara County and on the variety Fellenberg. During the season of 1931, the disease was found in 4 orchards affecting from 1 or 2 trees to 18 per cent in one small block of 58 trees. In 1934 a single diseased tree was found in another orchard. On one farm, trees of cherry, peach, and plum were growing within 30 feet of a severely affected prune tree, but no symptoms suggestive of a virus disease were seen on these. According to accounts of growers who report having had affected trees under observation for as long as 19 years, the disease seems to spread very slowly in the orchards of this district. Notwithstanding this fact and the small total number of trees now known to be involved, the disease finally renders the tree virtually worthless and constitutes a menace to prune culture in areas that may afford a more effective agent of dissemination.

Symptoms. The affected leaves are reduced in size and distinctly narrowed in proportion to length. Serration and pubescence are suppressed, and there is considerable rugosity and mottling (Fig. 1, B). The rugosity is more marked near the midrib than toward the margin of the leaf. The leaf blade is somewhat thickened. Leaf margins are frequently so irregular in outline as to resemble, under casual observations, the effect of chewing insects. The surface of the severely affected leaf presents a somewhat glazed aspect. All the leaves on a given shoot are affected, those at the base somewhat more so than those at the tip. The shoot may grow for several inches in a season or only a fraction of an inch. Internodes are shortened in varying degrees. An unusual feature of this disease is the development of a shoot that remains perfectly normal in appearance throughout the season, in the midst of buds that produce only severely affected foliage. Larger branches may be apparently normal and fruitful on trees that, otherwise, appear to be entirely involved by the disease; but this may be due to the fact that the virus has not yet invaded the entire tree. Diseased trees may blossom profusely, but only an occasional fruit matures on the parts that exhibit foliage symptoms. Pistils are aborted in many of the blossoms and petals are somewhat narrowed and irregular in shape (Fig. 1, B). The time of blossoming and of maturity of fruit seems not to be influenced by the disease, nor does the quality of the few fruits that reach maturity.

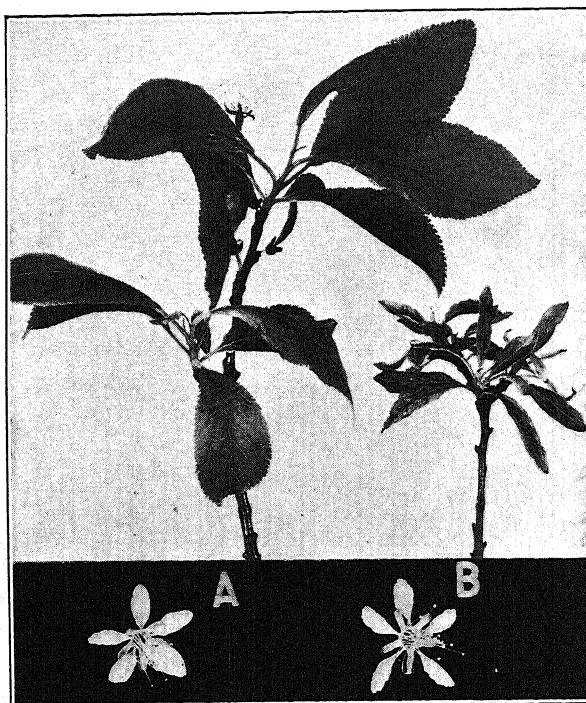


FIG. 1. A. Healthy prune leaves and blossom. B. Naturally infected prune branch and blossom from a diseased branch.

Infection experiments. On January 21, 1931, 10 cions from affected Fellenberg trees were grafted on 3 two-year-old German prune trees and these were planted at once in the greenhouse. Two trees of the same lot were planted as controls. Only 2 of the cions lived long enough to develop foliage, but symptoms appeared at or near 6 of the 10 points of inoculation. These were first seen nearly 60 days after inoculation, and at that time appeared only as a vague mottling in the leaf blade. Later, on some leaves, there appeared a very definite mottling and, on others, a distinct line pattern. Except on 1 of the 2 surviving cions, the typical narrowed and dwarfed leaves seen in the orchard were not produced in the first growth cycle. Shoots from buds more remote from the points of inoculation grew until August, 1931, some of them reaching 3 feet or more in length. Several of these appeared to be normal in all respects, while the leaves of others became distinctly rugose and mottled.

On May 21, 1931, buds from an infected shoot were set into 2 larger branches of a 15-year-old Fellenberg tree in the orchard. No infection was apparent on this tree on September 18, 1931. The abundance of red mites on the foliage at this time, however, would have obscured any but the more obvious symptoms. This tree was removed in 1932. In September, 1931,

John Goodrich inoculated a 22-year-old Fellenberg tree on the same farm by budding on a single terminal shoot. Symptoms were apparent on the inoculated branch in 1933, and, in 1934, they had spread only to about 1 foot below the point of inoculation.

Another experiment was begun on May 22, 1931. Buds from an affected prune tree were set into the following nursery trees: 2 cherry (Montmorency), 1 peach, 2 plum (Lombard), 2 prune (known locally as Smith's prune). Two buds were set into each tree in vertical line and covered with paraffin. The trees were then planted on the University Farm at Ithaca. One tree each of cherry, plum, and prune were planted as checks. None of the buds survived long enough to produce new leaves. The trees set in poor orchard soil made a short growth cycle followed by a brief second cycle during the latter part of the summer. No certain symptoms were seen on any of these during 1931. However, in July, 1932, definite symptoms were found on the inoculated prune and plum trees, resembling those of naturally infected orchard trees. The symptoms had become severe on the prune trees in October, 1934, and were marked on the plum trees. In 1935 the plum trees bore approximately a normal crop of fruit, while the prune trees bore less than one-fourth of a crop. The check trees and the inoculated cherry and peach trees remained healthy in appearance.

The preceding experiment was repeated in 1932 with essentially the same results. Inoculations were made in early August, 1932, and symptoms began to appear in early July, 1933. Again, prune and plum became infected, while cherry and peach did not. Further attempts are being made to transmit the disease to peach.

In a single experiment, 8 colonies of the green plum aphid (*Myzus Mahaleb*) were transferred from infected shoots and confined in cellophane bags on healthy prune and plum shoots. In another test, 4 colonies of *Aphis setariae* were similarly transferred from diseased to healthy shoots. In these trials, started in May, 1933, no infection resulted up to October, 1934, when the last observation was made.

The virus seems to spread rather slowly through the inoculated tree, as is the case with many virus diseases of woody plants. In this instance, judging by the time and place of the appearance of symptoms, the virus seems to move rather rapidly upward in line with the point of inoculation, distinctly less rapidly downward, and very slowly laterally.

RELATION TO DISEASES OF PRUNE AND PLUM IN OTHER AREAS

The locality (western New York) in which this disease is found is well beyond the present known range of plum rosette¹ and plum mosaic.² Moreover,

¹ McClintock, J. A. Peach rosette, an infectious mosaic. Jour. Agr. Res. [U. S.] 24: 307-315. 1923.

² Valleau, W. D. A virus disease of plum and peach. Kentucky Agr. Expt. Sta. Bull. 327. 89-103. 1932.

the prune disease seems to differ from these in symptoms, is less severe on plum, and fails to affect the peach. One of the writers has seen leaf symptoms on Santa Rosa plum in central California very similar to those shown for plum mosaic in Kentucky (Page 95, fig. 23).² In this (California) instance there was no noticeable reduction in size of leaves or in vigor of the trees 2 years after the symptoms were first seen. Symptoms resembling those of the prune disease have been described for prune in Holland,³ but are said to appear also on cherry and peach. No inoculation experiments are reported. No relation is apparent between the prune disease in New York and the group of diseases discussed by Atanasoff under the title "Mosaic disease of drupaceous fruit trees."⁴

Given little (or nothing) beyond symptoms as a basis for comparison, and these in some cases not fully described, it cannot now be determined with finality whether the disease under discussion is distinct from all those previously described. It seems unlikely, however, that this disease will prove to be identical with any thus far studied in plum or prune in North America.⁵

Under the observed conditions of limited incidence and slow rate of spread the disease should be amenable to control by eradication.

DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY AND
DIVISION OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA.

³ Rietsema, I. J. Weinig bekende ziekten in kers, pruim, en perzik. Tijdschr. Plantenziekten. 36: 261-269. 1930.

⁴ Atanasoff, D. Mosaic disease of drupaceous fruit trees. God. Sofisk. Univ. Agron. Lesov. Fakult. (Ann. Univ. Sofia, Facult. Agron. et Sylvic.) Livre I. Agron. 13 (1934/1935): 9-42. 1935.

⁵ There are now probably 4 virus diseases of plum and prune in the United States in addition to the peach diseases of "Yellows" and "Rosette," which also affect plums. In an unpublished paper presented by Donald Cation at the 1935-1936 A. A. S. meetings in St. Louis, entitled "A rosetted-mosaic of peach and plum trees," evidence on a new virus disease in Michigan was presented. This disease appears to be distinct from "peach mosaic" found in Colorado and "peach rosette" in the south. The junior writer was shown peach trees severely affected with rosetted-mosaic by Mr. Cation in 1933. According to Mr. Cation it usually is masked in the European plum varieties, but Burbank and Damson trees are very susceptible, showing a striking loss of vigor. Working with material similar to that of Valteau, Cation also has obtained evidence that Valteau probably was dealing with two plum viruses, one of which transmits to peach trees and causes a striking leaf pattern, while the other does not.

ENDOXEROSIS OF LEMON FRUITS AS AFFECTED BY THE APPLICATION OF DIFFERENT AMOUNTS OF IRRIGATION WATER¹

E. T. BARTHOLOMEW²

(Accepted for publication March 30, 1936)

Endoxerosis³ is an internal, nonpathogenic disorder, which appears in many lemon fruits as they approach or reach maturity. The first symptoms appear in or near the terminals of the vascular bundles in the peel at the stylar end of the fruit. As the disorder spreads, it may be found in isolated areas in any portion of the albedo of the peel. Concurrently, it begins to appear in the pulp at the stylar end and gradually spreads toward the stem end of the fruit. Gum is usually in evidence and the affected tissues show a pinkish yellow to a rust brown discoloration. Many of the affected cells, especially in the pulp, lose their water and disintegrate or collapse, leaving air spaces; hence the term endoxerosis (internal drying). By the time one-third to one-half of the pulp tissues are affected, the fruit usually falls from the tree.

Endoxerosis of lemon fruits has been reported from Sicily by Savastano (9) and from Australia by Jarvis (8). However, the symptoms for endoxerosis in these countries do not entirely agree with those for California.

For a more complete discussion of the distribution, characteristics, and contributing causes of endoxerosis, see Bartholomew, Barrett, and Fawcett (1), Bartholomew (2, 3, 5, 6), Bartholomew and Robbins (4), and Fawcett and Lee (7).

Previous observations and experiments had indicated that endoxerosis of lemon fruits might be largely influenced by the excessive withdrawal of water from the fruits by the leaves. Such a withdrawal would occur during periods of comparatively high temperature and low relative humidity in late spring and during the summer months. The experiment described in this paper was made in order either to disprove or substantiate this possibility.

MATERIALS AND METHODS

Twelve Eureka lemon trees, *Citrus limonia* Osbeck, budded on sour-orange stock, *C. aurantium* Linn., were used in this experiment. These

¹ Paper No. 342, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

² The author wishes to acknowledge his indebtedness to those who assisted in the experimental work, and especially to Mr. E. C. Raby, who made most of the soil-moisture determinations and gave other valuable assistance.

³ The term "endoxerosis" replaces "internal decline," the term used in earlier publications.

trees were grown in lysimeter tanks buried in the ground. The tanks were 10 feet in diameter, 4 feet deep at the edge, and 4.5 feet deep in the center. A detailed account of the special attention given to tree propagation, soil selection, soil-moisture determinations, and water applications will not be given here. These details are being published elsewhere.

The trees were planted in May, 1927, and from then to the end of 1934 all trees received the same amounts of pruning, soil fertilizers, and cultivation. In May, 1931, tests showed the apparent specific gravity of the soil in all tanks to be 1.5, the moisture equivalent approximately 11 per cent, and the wilting point 3.5 to 4 per cent.

The trees were divided into 3 groups of 4 trees each. The grouping was based upon the total production of endoxerotic fruits by each individual tree between January, 1929, when fruit production began, and May, 1931. During this period all trees received like amounts of water.

A different amount of water was applied to each group of trees between June, 1931, and December, 1934. Water was applied when the total mass of soil in each tank had reached an average moisture content of 6 to 8 per cent in group 1, 5 to 6 per cent in group 2, and 4 to 5 per cent in group 3. Sufficient water was applied to the soil in each tank at each irrigation to bring its entire mass up to an average moisture content of 11 per cent. The water was measured in and applied from a calibrated reservoir adjacent to each tank. The total number of gallons of irrigation water applied was 47,677 to the trees in group 1; 44,920 in group 2; and 38,545 in group 3.

Rainfall, measured in a rain-gauge that stood near the experimental group of trees, was taken into consideration in applying irrigation water.

Most of the fruit was picked according to regular ring-size method, which means that each fruit had attained a transverse diameter of at least 2.25 inches before it was picked. However, endoxerotic, windfall, tree-ripe, and sunburned fruits that did not attain full ring size were included in the tree yield. Total individual tree yields were also weighed at each picking.

RESULTS

The principal results obtained in this experiment are shown in table 1. The data in the table show that before differential water treatments were begun, the fruit production of groups 1 and 3 was practically the same, while that of group 2 was somewhat less. While the total production of good fruit over the entire period from 1929 to 1934 was greatest for group 1, the increase in yield during the second period (period of differential water treatment) was greatest for group 2. The increases in yield of good fruit for the second period were 575, 676, and 190 pounds, respectively, for groups 1, 2, and 3.

TABLE 1.—Proportions of good and endoxerotic fruit produced by experimental tree groups, 1929-1934, inclusive^a

Group	1929-31			1932-34				
	Condition of fruit			Soil moisture content ^b	Irrigation water applied	Condition of fruit		
	Good	Endoxerotic				Good	Endoxerotic	
	<i>pounds</i>	<i>pounds</i>	<i>Per cent</i>	<i>per cent</i>	<i>gallons</i>	<i>pounds</i>	<i>pounds</i>	<i>Per cent</i>
1	1,389	137	9	6-8	47,677	1,984	245	11
2	1,219	151	11	5-6	44,920	1,895	211	10
3	1,414	106	7	4-5	38,545	1,604	328	17

^a Differential water treatments were begun in June, 1931.

^b The figures in this column indicate the amount of moisture in the soil each time that irrigation water was applied. For the period 1929-31, all trees received like amounts of water.

During the period over which all groups received equal amounts of water, group 2 produced the greatest percentage of endoxerotic fruits and group 3 the least. During the second period (1932-34), group 2 produced about the same percentage of affected fruits as during the first period, while there was a slight increase in group 1, and a comparatively marked increase in group 3.

A single picking on August 6, 1934, showed results similar to those for the 1932-34 period in table 1. In this picking, 561 of the fruits from the trees in group 1, 459 from group 2, and 1,915 from group 3 were endoxerotic. These figures represent 40, 37, and 62 per cent, respectively, of the fruit picked from the 3 groups on that date.

It is of interest to note also that the sizes of the endoxerotic fruits for the period 1932-34 were smaller for group 3 than for either of the other 2 groups. For example, during the period 1931 to 1934 it took an average of 7.3 endoxerotic fruits from the trees in group 1, 6.6 from group 2, and 8.5 from group 3 to weigh 1 pound.

DISCUSSION

That there is an element of danger in attempting to interpret results obtained from as small a population as only 12 trees, 4 in each group, is fully realized. However, it should be remembered that the trees were carefully propagated from known sources and were grown under controlled conditions; the group differences in these tests, though not large, are positive; and the results confirm those of previous tests and observations. Such factors make the conclusions much more reliable than if the trees had been grown and cared for under the usual field conditions.

The difference shown in table 1 between 11 per cent endoxerotic fruits for group 1 and 10 per cent for group 2 for the second period is negligible, but it becomes more significant when compared with 9 per cent and 11 per cent, respectively, for the first period of the treatment. It becomes even more significant when compared with the results obtained in the specially mentioned pick of August 6.

As shown in table 1, the production of good fruit in group 1 went from second place in the first period to first place in second period, while group 2 went from third place to second place. Based on production during the first period, however, not only the yield of good fruit, but also the total yield of group 2 exceeded that of group 1 during the second period. These results indicate that the greatest yield of good fruit may be obtained when a moderate amount of irrigation water has been applied and that this maximum yield may be obtained without the appearance of any more, and possibly less, endoxerosis.

Many fruits attain full size before showing an endoxerotic condition. However, that many of the fruits may be affected before they become mature is shown by the fact that during the period 1931 to 1934 an average of 7.3, 6.6, and 8.5 endoxerotic fruits from groups 1, 2, and 3, respectively, were required to make 1 pound in weight. The fact that for the pick of August 6, 1934, it took 9.4 endoxerotic fruits from group 3 to weigh a pound indicates in a marked way that a deficiency of soil water causes smaller fruits to be affected. These fruits may have been small because they were young or because they were slow-growing and stunted. It takes an average of 4 fruits of the usual picking size to weigh 1 pound.

The results reported in this paper indicate that the development of endoxerotic conditions in lemon fruits is influenced by the withdrawal of water from the fruits by the leaves during periods of excessively warm weather. However, the withdrawal of the water from the fruit by the leaves is not the only factor concerned. A comparatively high temperature, aside from its effect on the rate of transpiration, is necessary. Furthermore, the presence of substances that may be converted into gums, hexosans (4), appears to be an important factor. It would seem that a little more of these substances was produced in the fruit of group 1 and much more in the fruit of group 3 than in that of group 2. This agrees with the previous observations that young, thrifty trees with a comparatively large unit of transpiring surface per fruit produce more endoxerosis than those with a moderate amount of foliage; but the greatest amount of endoxerosis is found on trees that over a period of years have not had enough water.

The experimental results indicate that the withholding of water from lemon trees until the moisture content of the soil had almost reached its

wilting point produced cumulative effects in the trees and fruit. This is shown by the fact that, although the trees in group 3 had received an irrigation that brought the soil mass in each tank up to its full moisture equivalent at the beginning of the hot period of 7 days, the pick made from this group, 4 days after the close of the hot period, showed 62 per cent of the fruit to be endoxerotic. Incidentally, the amount of endoxerosis has been observed to increase from summer to summer after a series of low winter rainfalls.

SUMMARY

Three groups of trees were grown in tanks in soil having a moisture equivalent of 11 per cent and a wilting point of 3.5 to 4 per cent. During the first period of the experiment the trees received like amounts of water, while during the second period they were not irrigated until the soil mass in the tanks had reached a moisture content of 6 to 8 per cent in group 1, 5 to 6 per cent in group 2, and 4 to 5 per cent in group 3.

The trees in group 3, which received the fewest irrigations, and the least amount of water, produced the greatest quantity of endoxerotic fruit and the greatest number of fruits that became endoxerotic before they reached picking size.

During the period of differential water treatment the trees receiving the medium amount of water produced the greatest amount of good and the least amount of endoxerotic fruit.

The results of these tests substantiate previously obtained evidence to the effect that the withdrawal of water from the fruit during periods of excessively high temperature and low relative humidity is an important factor in the production of endoxerosis.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

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PHYTOPATHOLOGICAL NOTE

Spore Formation and Discharge in Fomes fomentarius.—In March, 1930, at the Laboratory of Phytopathology of the Central Forest Experiment Station (Moscow, U. S. S. R.) there was obtained a green block of lime wood seriously injured by the fungus *Fomes fomentarius*. On the surface of the block there were two well-developed fruit bodies of the fungus. The block of wood was so placed on a stand in the corner of the room that one of the fruiting bodies, situated at the top, faced the window, while the other, situated considerably below on the other side, was 12 cm. from the pipes of the central heating system.

Presently there appeared on the lower surface of the fruiting body of the fungus a thin white layer, at first on the more heated side and afterwards over the whole surface.

Five or six days after the first signs of this phenomenon, the entire fruiting body of this fungus was covered with a thick layer of white spores. They were oblong, measuring $10-18 \times 4-6 \mu$. The spores, on being discharged from the hymenium, apparently were drawn upwards by the movement of the heated air and precipitated on the colder surface of the fruiting body.

Spore discharge of *Fomes fomentarius* was observed during approximately one month. The precise moment of their appearance was not determined. On March 25 they already covered the whole surface of the fruiting body and by April 15 their development was discontinued. It is possible that in nature spore formation proceeds more slowly than under laboratory conditions, where the gradual drying of the wood doubtless inhibited their further development.

The spores were collected abundantly every day and weighed. From March 25 until April 13 there were collected a total of 1,115g. This figure does not represent the total amount, as at the outset the spores were not collected and naturally some of them may have been carried away by the movement of the air. Others may have been lost while collecting.

In order to determine the approximate number of spores discharged during the time of their collection (20 days), they were computed as follows: The average size of a spore is $14 \times 5 \mu$; assuming the shape of a spore as an ellipsoid, it is possible with the help of a formula of rotation of an ellipsoid around its large axis to find the volume of one spore: $V = 4/3\pi b^2 \cdot a$ (a—a large, b—a small semiaxis). Its volume equals 163 cb. μ . Assuming then that the specific gravity of one spore is approximately that of water and may be taken as 0.9, we find the weight of one spore. It is equivalent

to 0.000,000,000,146 gr. Dividing the $\frac{1,115}{0.000,000,000,146}$ we get the number of spores discharged within 20 days, that is from March 25 until April 13. This number is equivalent to 7,563,493,150. During a 24-hour period the single fruiting body of *Fomes fomentarius* under observation discharged 378,424,655 spores.

The above mentioned computations roughly approximate the truth, but they, nevertheless, indicate the probable number of spores discharged by a single fruit body of *Fomes fomentarius*.

The pure culture consisting of collected spores of *Fomes fomentarius* had the usual macroscopic appearance, i.e., a continuous felt-like mycellium, smooth, at first yellowish, then brown. Growth of culture is moderately rapid. Among the hyphae of the aerial mycelium there are mostly fiber-like 1-4 μ in diam., thick-walled, poorly ramified, continuous hyphae, often a golden chestnut color.

The hyphae of the submerged mycelium have thin walls and are 2-7 μ in diam. with strands, continuous, ramified.

The spores possessed a high capacity for germination. When sown in the Petri dish on agar-malt medium, they formed on the next day an abundant growth of mycelium. They germinated rather well on the water-soaked block of wood. The germinating power of spores is of a rather long duration. The spores kept in the laboratory in cotton-plugged test tubes germinated after 5 months as well as newly collected spores. Tested in March, 1931, one year after collection, they showed 25 per cent of germinating power.—HELEN MEYER, Centr. Forest Experiment Station, Moscow, U. S. S. R.

BOOK REVIEW

Raillo, A. I. Diagnostic estimation of morphological and cultural characters of species in the Genus *Fusarium*. [In Russian with English title.] 100 pp. illus. Inst. Zashch Rast. Trudy Zashch. Rast (Leningrad Acad. Agr. Sci. U.S.S.R., Inst. Plant Prot. Bull. plant protect.) series 2 Phytopath., No. 7. Published by the Lenin Acad. of Agr. Sci., Leningrad, 1935. Price 3 rubls.

This interesting publication by Miss Raillo presents a decisive departure from the customary method of approach to the classification of the genus *Fusarium*. Heretofore, the author points out, no complete and proper evaluation of the various taxonomic characters, typifying different subdivisions of the genus, has been made and, consequently, no clear concept of the structure of species and its dynamics could have been formed. Certain features have been indiscriminately used for the differentiation of higher units in one case and lower in another. This confusion is regarded as the chief reason for the great difficulties that are encountered by practically everyone in attempting to identify or classify *Fusarium* cultures. These difficulties are further augmented by the fact that, as a rule, different workers have used different technique, and thus no comparison of results is possible.

Raillo's objects were: (1), to thoroughly standardize methods of procedure; (2) to estimate the diagnostic value of each character, and (3) to draw up a scheme of the structure of species and of its dynamics.

The standardized procedure employed by Raillo was as follows: 50 *single-spore* cultures were used for every taxonomic unit studied and on each of the 5 kinds of *media* employed (potato agar and acid potato agar for fruiting, rice and potato plugs for pigment and, in some cases, to check up on certain behavior, also the synthetic medium of Leonian). All cultures were held under a diffused light at the temperature between 22° and 25° C. *Measurements* of the conidia were made on the 15th day, provided the typical fruitification was present; otherwise, on the 30th or 45th day, etc., so that no spores were used older than 15 days; 100 conidia with the prevailing number of septae were measured in each culture and all measurements were treated statistically. *Drawings* were made at the time of *measurement*, with a uniform magnification of x 1000. The description of *pigment* development was made on the 15th and the 30th day, using Ridgway's system of color nomenclature and painting, on the 30th day.

No question is raised as to the validity and clearness of Wollenweber's characterization of the sections in the genus *Fusarium*. Moreover, the precise determination of the section to which a given *Fusarium* culture belongs, is considered to be the first and indispensable step in the identifica-

tion of this culture. The critical revision and revaluation concern those taxonomic features that characterize the species and its subdivisions. In particular:

Length and width of macroconidia. These characters occupy more or less dominant position in existing systems, but Raillo gives them only a subordinate value. A study of the amplitude of variations in the length of the conidia and of the ratio between the difference of means of two extreme rows and their mean error, convinces her that the length cannot serve as a diagnostic character for higher taxonomic units, unless the amplitude of variations is given. The length measurements of conidia of one isolate may characterize only this isolate, or at the most, the race. The width measurements, likewise, show unequal value of different conidia, and, therefore, are not regarded as distinguishing for higher taxonomic units unless the amplitude of fluctuations is given.

Septa. Wollenweber and other workers used the number of cross walls as a fundamental character in distinguishing species and varieties, but Raillo finds that too great variations exist, even for different isolates of the same single-spore cultures. She studied the distribution of the number of septa among 100 observations for each culture and concluded that only the prevailing number may be used for characterization of a taxonomic unit.

The *shape of the conidia* is a complex character and is determined by the three component parts: (a) form of the apical cell, (b) length of the apical cell, and (c) incurvation of the conidia. These three features are evaluated separately.

(a) *Form of the apical cell* has heretofore received very insufficient attention, but Raillo finds that this is the only constant character for all single-spore cultures of a given species and, therefore, diagnostic for the species within each section. The terms for the designation of shape variations of the apical cell are adopted from the Wollenweber's system, and are used to denote the manner and the degree of tapering. Accordingly, the following 7 types are recognized by the author: (1) slightly narrowed and rounded, as in *F. solani* (Mart.) App. et Wr. (section Martiella); (2) suddenly narrowed as in *F. culmorum* (W. G. Sm.) Sacc. var. *lethaeum* Sherb. (section Discolor); (3) gradually and evenly narrowed, as in *F. equiseti* (Cda.) Sacc. (section Gibbosum); (4) same as (3) but truncated, as in *F. lateritium* Nees var. *majus* Wr. (section Lateritium); (5) sharply and considerably narrowed, as in *F. scirpi* Lamb. et Fautr. (section Gibbosum); (6) filiform, as in *F. scirpi* Lamb. et Fautr. var. *filiferum* (Preuss.) Wr., and (7) same as (6) but crooked, as in *F. scirpi* Lamb. et Fautr. var. *caudatum* Wr.

(b) *Length of the apical cell* has diagnostic value only in those cases when its elongation changes the morphology of the conidia, which may be

seen in the case of gradually and considerably narrowed or filiform cells, as in *F. herbarum* and *F. avenaceum* (section *Roseum*). No generalization can be made on this basis, however, in regard to species in other sections without a further detailed study.

(c) *Curvature of the conidia* has been regarded by Wollenweber as a diagnostic character for varieties and forms. Raillo finds that it should be used as a distinguishing character for subspecies, since its dynamics indicate the variation within the species, but not within lower divisions. There are 5 known types of curvature for the designation of which Raillo uses terms introduced by Wollenweber, namely: (1) nearly straight, as in *F. semitectum* Berk. et Rav. (section *Arthrosporiella*); (2) eel-shaped, as in *F. diversisporum* (section *Arthrosporiella*); (3) elliptical, as in spp. of sections *Elegans*, *Sporotrichiella*, *Lateritium*, *Liseola* and others; (4) parabolic, as in *F. equiseti* (Cda.) Sacc. f. I Wr., and (5) hyperbolic, as in *F. scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr.

The author's general conclusion as regards cultural characters is that they are of taxonomic value, but for lower units only. Namely:

(a) *Pigment* on rice can characterize forms. On potato plugs and on the Leonian agar sharp differences occurred even among the single-spore isolates from one single-spore culture, showing that on these media pigment can characterize races only.

(b) *Sclerotia*, as regards their color and size, presence or absence, were found by Raillo to vary greatly even among different isolates and, consequently, characteristic of races only.

(c) The type of *fruiting* (sporodochia, pseudopionnotes and pionnotes) likewise is regarded as characteristic of races only.

The foregoing analysis tends to show that the *Fusarium* species is a complex system characterized by series of parallel and non-parallel features. Only one of them is found by the author to be of diagnostic value for the species, i.e., the form of the apical cell, as a non-parallel character for other species in a given section. It cannot be used as a sectional character, since it is not identical for all species in the majority of sections. The various elements of morphology of the conidia may become a basis for characterization of higher taxonomic units only in their dynamics and the systematic value of complex characters will vary in accordance with variations within the species. The author illustrates this idea by schematic structures of species having different variability.

An English summary is appended to each of the two parts of the book, but these summaries are too brief to be of practical guidance to those who may wish to test out the Raillo's methods. Hence the reviewer felt justified in presenting to the readers of *Phytopathology* this rather extensive abstract until a complete translation becomes available.

It is understood that the author is now engaged in the preparation of a monograph of the genus, which will embody the principles advanced in this preliminary paper. It is then, as well as through the tests that other workers may make in connection with their own *Fusarium* problems, that these principals will find their crucial test. At this moment no final judgment can be made as to the validity of the proposed method. However, certain conclusions advanced by Raillo are obviously sound and correct, particularly as regards the necessity for any *Fusarium* specialist, first of all, to estimate definitely the diagnostic value of various taxonomic characters and, subsequently, to build up a clear concept of the structure of species and the genus. It is also very evident that the deductions made from the variability data concerning the different morphological and cultural characters, are logical and point to the most confusing irregularity in their present usage. Whether the form of the apical cell is a constant character for the species in a given section can be determined only by a thorough analysis of all the known forms, but Raillo's affirmative answer may be accepted as correct as long as it does not contradict the facts. However, the terms defining the several varied forms of the apical cell in some cases leave greater precision and indisputable clarity to be desired. Thus, it would seem that such differences as exist between the types represented by *F. solani* on the one hand and *F. culmorum* var. *lethaeum* on the other, or between the apices of *F. equiseti* and *F. lateritium* var. *majus* are not clearly defined. It is possible that coinage of new terms will be necessary.

The book contains a considerable number of very excellent drawings of the types of macroconidia. It is quite regrettable that there are no colored plates illustrating the variability in pigment described by the author in the text. It is hoped, however, that they may be included in the forthcoming monograph.—MICHAEL SHAPOVALOV, U. S. Dept. of Agriculture, San Jose, California.